



Chemical Synthesis of Hemicellulose Fragments

Böhm, Maximilian Felix

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Chemical Synthesis of Hemicellulose Fragments

PhD Thesis

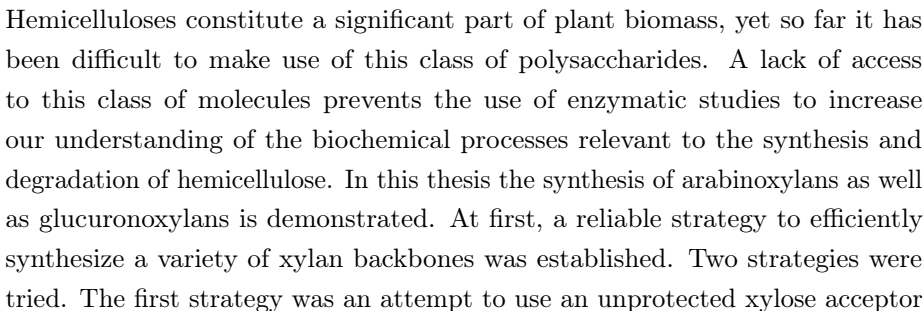
Maximilian Felix Böhm

DTU



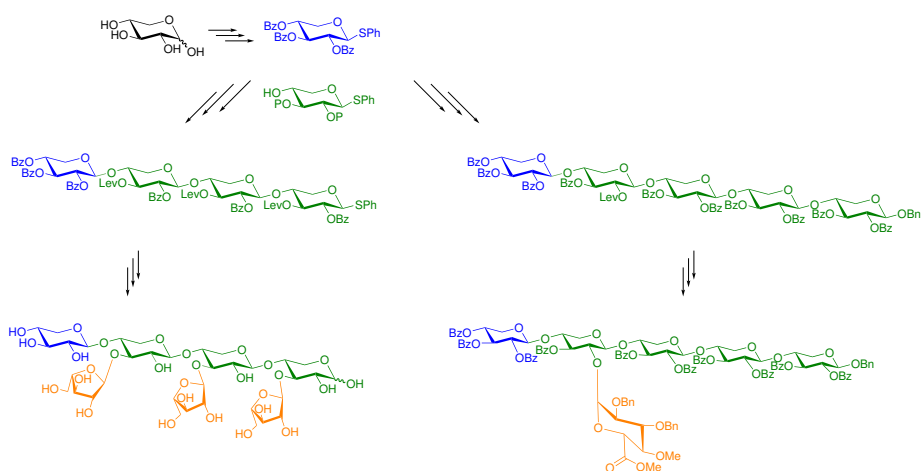
Kongens Lyngby 2016

Technical University of Denmark
Department of Chemistry
Kemitorvet, building 211,
2800 Kongens Lyngby, Denmark



in a tin-mediated glycosylation. Since the best results of the optimization of this reaction were not good enough a second strategy was pursued. This second strategy is based on the preactivation of thioglycosides to be glycosylated with thioglycoside acceptors which in turn can be preactivated again in a second step. Optimization of this strategy lead to a viable pathway towards a variety of protected xylan backbones. The use of protecting groups allows for the specific introduction of branching units to the backbone. Subsequently arabinose as well as glucuronic acid were attached to the xylan backbone.

Summary (Danish)



Hemicellulose udgør en væsentlig del af plantebiomasse, men alligevel har det været vanskeligt at få adgang til denne klasse af polysaccharider. En manglende adgang til denne klasse af molekyler forhindrer enzymatiske undersøgelser der vil kunne øge vores forståelse af de biokemiske processer relevant for syntese og nedbrydning af hemicellulose. I denne afhandling er syntesen af arabinoxylaner samt glucuronoxylaner demonstreret. Der blev etableret en strategi for en pålidelig og effektiv syntese af en række xylan skeletter. To strategier blev forsøgt. Første strategi var et forsøg på at bruge en ubeskyttet xylose acceptor i en tin-medieret

glycosylering. De bedste resultater under optimeringen af denne strategi var ikke gode nok og derfor blev en ny strategi forsøgt. Strategi nummer to blev baseret på præaktivering af et thioglycosid der senere hen glycosyleres med en thioglycosid acceptor som igen kan præaktiveres i et efterfølgende trin. Optimering af denne strategi førte til en brugbar metode og førte til en række af forskellige beskyttede xylan skeletter. Anvendelsen af beskyttelsesgrupper introducerer en specifik forgrening af enheder til skelettet og medførte efterfølgende at arabinose samt glucuronsyre blev bundet til et xylan skelet.

Acknowledgements

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Abbreviations

Ac	Acetyl
AcCoA	Acetylcoenzyme A
ACN	Acetonitrile
Ara	Arabinose
Ara <i>f</i>	Arabinofuranose
AX	Arabinoxylan
BAIB	[bis(acetoxy)iodo]benzene
Bn	Benzyl
BSP	1-Benzenesulfinyl Piperidine
Bz	Benzoyl
ClAc	Chloroacetyl
CIP	contact ion pair
CSA	Camphor-10-sulfonic acid
CSL	Cellulose synthase-like
d	Doublet
DAST	Diethylaminosulfur trifluoride
DCC	<i>N,N'</i> -dicyclohexylcarbodiimide
DCM	Dichloromethane
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DMAP	4-Dimethylaminopyridine
DME	Dimethoxyethane

DMF	<i>N,N</i> -Dimethylformamide
DMSO	Dimethyl sulfoxide
DMTST	Dimethylthiomethylsulfonium triflate
dt	Doublet of triplets
Et	Ethyl
<i>f</i>	furanose
Fmoc	Fluorenylmethyloxycarbonyl
GH	Glycosyl hydrolase
GlcA	Glucuronic acid
GUX	Glucuronosyltransferase on xylan
GDP	Guanosine diphosphate
GXMT	Glucuronoxylan transferase
HMBC	Heteronuclear Multiple Bond Correlation
HPLC	High performance liquid chromatography
HRMS	High resolution mass spectrometry
Lev	Levulinoyl
m	Multiplet
Me	Methyl
MeGlcA	4- <i>O</i> -Methyl glucuronic acid
MS	Molecular sieves
NBS	<i>N</i> -Bromosuccinimide
NIS	<i>N</i> -Iodosuccinimide
NMR	Nuclear magnetic resonance
<i>p</i>	pyranose
Ph	Phenyl
PMB	<i>para</i> -Methoxybenzyl
<i>p</i> -TSA	<i>para</i> -Toluenesulfonic acid
Py	Pyridine
R_f	Retardation factor
RGP	Reversibly Glycosylated Protein
r.t.	Room temperature
s	Singlet
t	Triplet
TEMPO	(2,2,6,6-Tetramethylpiperidin-1-yl)oxy
Tf	Trifluoromethanesulfonyl

TFA	Trifluoroacetic acid
TfOH	Trifluoromethanesulfonic acid
TLC	Thin layer chromatography
TMS	Trimethylsilyl
TMSCl	Chlorotrimethylsilane
TTBP	2,4,6-Tri- <i>tert</i> -butylpyridine
UDP	Uridine diphosphate
UGE	UDP-Glc Epimerase
UXE	UDP-Xyl Epimerase
UXS	UDP-Xyl Synthase
XAT	Xylan arabinosyl transferase
Xyl	Xylose

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CHAPTER 1

Introduction

1.1 The Cell Wall

In 1804, the Royal Society of Science in Göttingen (Königliche Societät der Wissenschaften) publicly issued a competition to answer the question of how plant cells are built. Of special interest at that time has been the question of whether plant cells have individual walls or whether there is only one wall encompassing all cells. The question was answered by Friedrich Heinrich Link in Rostock and simultaneously by Karl Asmund Rudolphi in Greifswalde. In 1807, Link published their findings in a text book on plant anatomy and physiology (Figure 1.1).¹

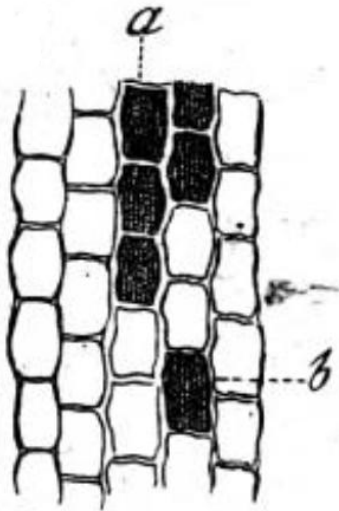


Figure 1.1: Microscopy sketch by Link of *Lyfimachia thyrfiflora*, clearly showing the cell walls.¹

Since then, many details about the cell wall's structure, composition and function have been discovered. Nowadays, it is generally divided into two parts: primary and secondary cell wall. The primary cell wall is recognized as a thin and pliant structure, that allows for the growth of the cell. The mature plant then develops a secondary cell wall, which is thicker, stronger and more rigid to provide stability.² Keegstra notes in his review on plant cell walls that this

division applies to two extreme states, since all cells have their own distinct, specialized cell walls.³ Chemically, cell walls are comprised of a host of different macromolecules - proteins, lignin and polysaccharides.

The fact that most proteins in the cell wall are glycosylated and that the cell wall consists mostly of polysaccharides as well as the difficulty of eliminating contaminations, has hampered the process of identifying cell wall proteins to a great extent. Nonetheless, it is known that there are many proteins involved in the building and modification of the cell wall and cell signaling, often times related to defense and (a)biotic stress.⁴ They also enable plants to be strong as well as flexible.²

Lignin is a phenolic polymer that gives plants mechanical support to stand upright and to enable the xylem to withstand the negative pressure, allowing for water transportation. Lignin-deficient mutants have shown diminished growth and sometimes dwarfing, indicating the importance of lignin in stabilizing the xylem.⁵

The best known cell wall polysaccharide is cellulose, which is an integral part of every plant's cell wall. After synthesis in the cytosol and transport through the membrane, cellulose chains form crystalline microfibrils via hydrogen bonding, thereby contributing to the cell's rigidity.⁶

Pectin constitutes the second class of polysaccharides of the cell wall. Pectin contains several different polysaccharide domains that are covalently connected with each other. The main pectic polysaccharides are homogalacturonan, xylogalacturonan, apiogalacturonan, rhamnogalacturonan I and rhamnogalacturonan II.⁷ Pectin contributes to a plant's strength as well as flexibility, and forms part of a barrier against pathogens. Additionally, it has been shown that pectin fragments interact with kinases that are involved in stress and pathogen response pathways.⁸ This allows plants to react accordingly to damage and attacks. Pectin is synthesized in the Golgi in its methylated form and de-esterified upon release into the cell wall.⁸

The third class of polysaccharides are hemicelluloses. First described in 1891 by Ernst Schulze⁹ as "those components of the cell wall that are easily soluble in hot, diluted mineral acids",¹⁰ the term has undergone a change in meaning. Hemicelluloses are nowadays defined as a group of polysaccharides, characterized by being neither cellulose nor pectin and by having β -(1 \rightarrow 4)-linked backbones of glucose, mannose or xylose.¹¹ This class will be further discussed in the next section.

The plant cell wall is a highly sophisticated system of macromolecules that fulfills a multitude of functions, necessary for a plant's life. Models of the structure and composition of this essential cell component have been growing more complicated over the years (Figure 1.2).

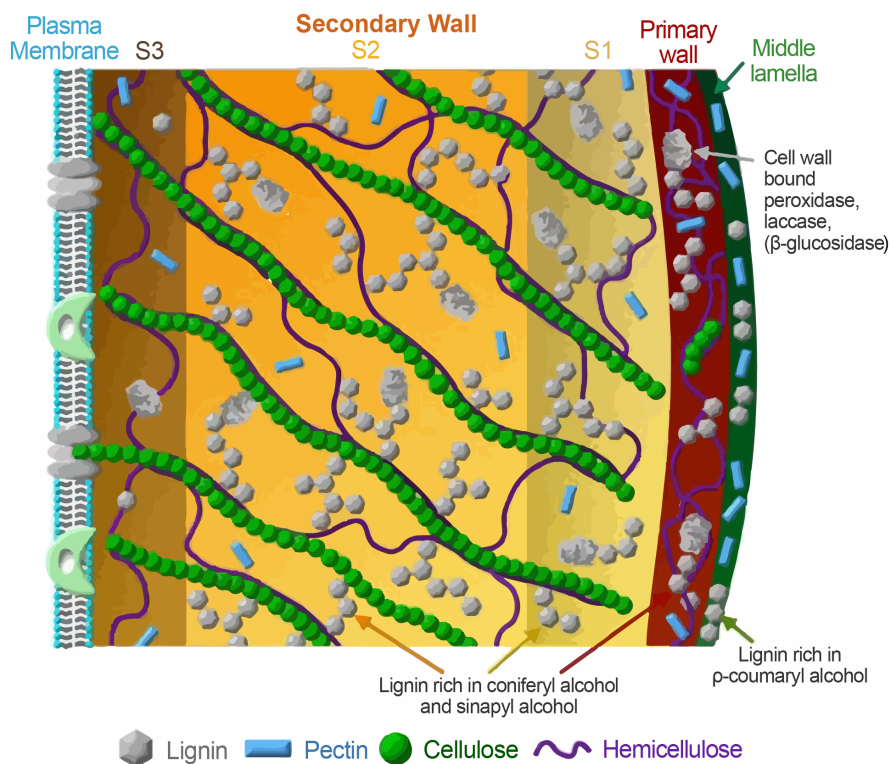


Figure 1.2: A modern depiction of cell wall composition, preserving the relative size of the different layers as well as abundance and localization of the various components¹²

An increase in knowledge has led to an increase in application. The abundance of plants makes their utilization attractive. While many applications have been found in the energy sector,¹³ food¹⁴ and materials^{15,16} there remains room for improvement. Pentoses, for example, are not fermented by yeast and are therefore inaccessible to normal ethanol production.¹⁷ Since pentose containing hemicelluloses account for up to 50% of the biomass of annual and perennial

plants,¹⁸ a lot of material goes unutilized. Isolation as well as application of non-cellulosic cell wall components remain challenging. An increased understanding of the biosynthesis and degradation of these compounds, would allow scientists to make better use of this abundant and renewable resource.

1.2 Hemicelluloses

According to the previously mentioned definition, hemicelluloses are a group of polysaccharides having β -(1 \rightarrow 4)-linked backbones of glucose, mannose or xylose.¹¹ From this definition, there emerge three distinct classes, two of which have their own subclasses:

Mannans mannans, galactomannan, glucomannan and galactoglucomannan

Xyloglucans This class has been found to be sometimes covalently linked to pectin and non-covalently binding to cellulose^{19–21}

Xylans glucuronoxylans (GX), glucuronoarabinoxylan (GAX), arabinoxylans (AX)

Among the various hemicelluloses, xylans are the most common, constituting the second most abundant biopolymer in the plant kingdom.²² All classes have been found to be acetylated to some degree in their native state.²² Additionally, ferulic and coumaric esters can be formed linking the hemicellulose to lignin.¹¹

Generally, hemicelluloses contribute to the strength of the cell walls and are of special relevance in secondary cell walls. This often puts them in second place after cellulose in plant biomass. Xylans have been shown to be critical in the growth of some plants, thereby indicating a potentially important role in stabilizing the vessel walls necessary to transport water. The vessel walls need to be strong enough to withstand the high negative pressure generated by transpirational pull.¹⁴

In seeds, hemicelluloses can function as storage carbohydrates, analogous to starch.¹¹ This has been shown to be the case for xylans in the endosperm of cereal grains.

Hemicelluloses are able to bind to cellulose microfibrils within the cell wall (Figure 1.2).²² Apart from its stabilizing properties, arabinoxylan has been shown to inhibit ice formation and might contribute to the survival of plants during winter.²³

Hemicelluloses occur in many varieties depending on the plant and the part of the plant examined. This abundance makes them attractive targets for utilization, for example in the food industry influencing the quality of cereal flours and dough or as a starting material for xylitol, a popular sugar substitute.²⁴

Another major target application can be found in the conversion of biomass to bioethanol. First generation biofuels are produced through fermentation of glucose derived from starch. One of the main disadvantages consists in the competition of food-crops with fuel-crops. To circumvent this problem, second generation biofuels are currently being developed where glucose is obtained from cellulose.¹³ The aim here is to utilize the non-food plant biomass, which consists mainly of lignocellulosic material. Potential resources could be the pulp and paper industry, various crops, straw and grass.¹⁸ The difficulty of this approach lies in the inherent stability of lignocellulosic biomass. Lignin is cross linked with cellulose and hemicellulose. Additionally, a large percentage of hemicellulose consists of xylose and other pentoses, which are currently difficult to ferment. This creates a need for the development of processes that can extract the fermentable sugars as well as increasing the amount of fermentable sugars and to apply these processes to all parts of a plant.¹³

One component in this development might be the genetic modification of plants, which in turn would ease the efforts of extraction and conversion of useful components. Potential approaches would target a decrease of the acetate content which inhibits the fermentation by yeast or a modification of the ferulate esters to reduce the attachment to lignin.¹⁷

1.3 Xylans

1.3.1 Structure

Arabinoxylans consist of a β -(1 \rightarrow 4) connected xylopyranose backbone with L-arabinofuranose substituted on the 2-and/or 3-positions (Figure 1.3). At least in grasses, the O-3 position of the xylose residue seems to be generally preferred for Araf substitution.¹¹

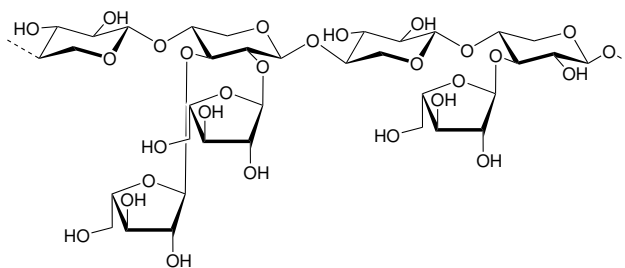


Figure 1.3: Chemical structure of L-arabino-D-xylan

Additionally, it has been found that in grasses, the 5-position of arabinose is substituted with ferulic acid, linking it to lignin.^{17,24}

In glucuronoxylans glucuronic acid is typically α -(1 \rightarrow 2)-linked to the xylan backbone. Often times, the glucuronic acid is methylated on the 4-position (Figure 1.4). According to a 2012 study, this might enhance the overall stability of the cell wall and an artificial decrease in O-4 methylation leads to to an increased release of glucuronoxylan upon mild hydrothermal pretreatment.²⁵ Some xylans have been shown to contain arabinose as well as glucuronic acid - so called glucuronoarabinoxylans (GAX).

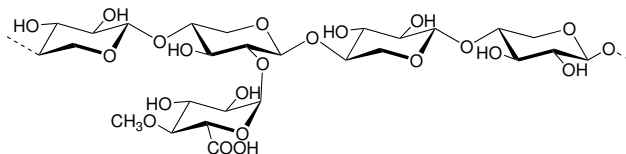


Figure 1.4: Chemical structure of Glucuronoxylan

In 1971, xylan hydrate has been found to form twisted ribbon-like strands with three-fold symmetry winding around a column of water which differs from mannan and cellulose chains that have been found to form untwisted, ribbon-like structures with a two-fold symmetry. Addition of L-arabinose units do not change the basic conformation of the xylan structure (Figure 1.5).^{26,27}

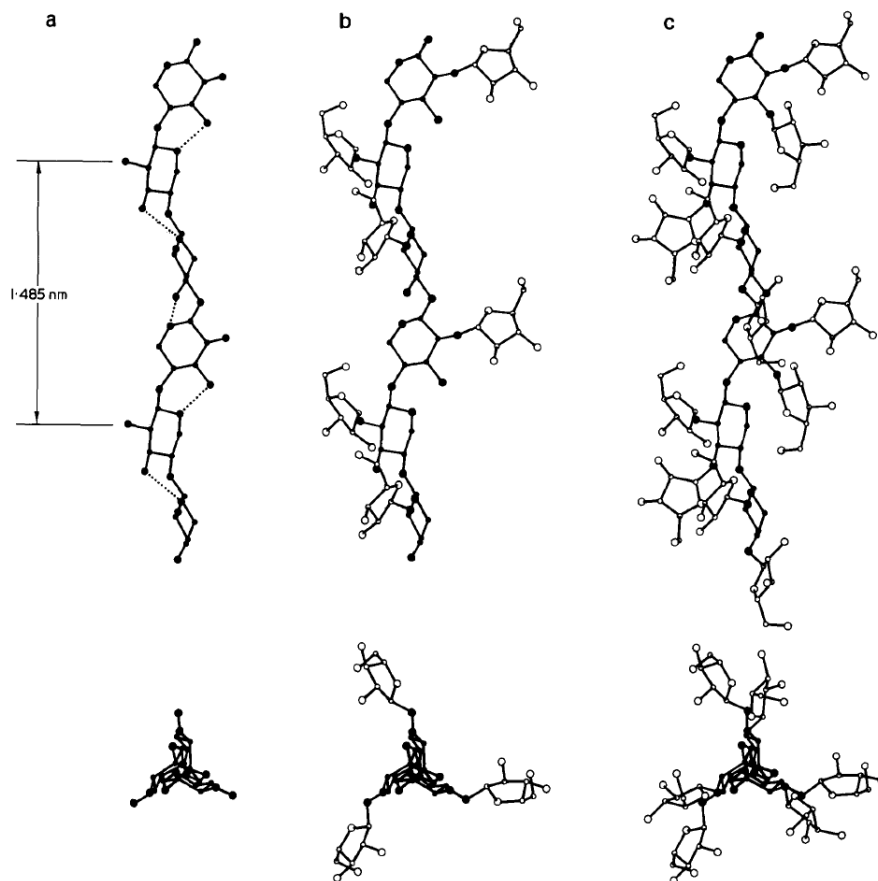


Figure 1.5: Projections perpendicular (top) and parallel (bottom) of (a) xylan backbone, (b) backbone and one L-arabinose side group; (c) backbone and two L-arabinose side groups. Hydrogen bonds are shown dotted. In each case the backbone is a left-handed three-fold helix.^{27,28}

1.3.2 Biosynthesis

An overview of the biosynthesis of xylans and other hemicelluloses has been most recently given by Pauly et al. in 2013¹⁴ as well as Rennie and Scheller in 2014.¹⁷

Unlike for mannans and xyloglucans, no cellulose-synthase-like (CSL) genes have been identified in the backbone synthesis of xylans. Instead, a variety of glycosyltransferases (GTs) of the GT43 family have been found to be involved in this process: IRX9, IRX14, IRX10 in *Arabidopsis*. These GTs are named after irregular xylem phenotypes of their corresponding mutants, affected by dwarfism. It has been observed that abundance and length of xylans in these mutants is reduced. Similar genes have been identified in poplar.

Glycosidic linkages are made by GTs utilizing activated donors (usually the UDP or GDP derivatives, but also nucleotide mono phosphates, lipid phosphates and unsubstituted phosphates).²⁹ In the case of the xylan biosynthesis, the UDP-derivatives have been identified as the enzyme substrates:

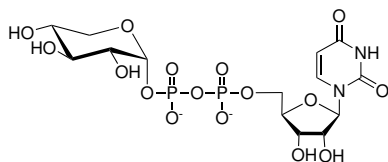


Figure 1.6: UDP-Xylose is used as the the substrate for glycosyltransferases in the xylan synthesis.

UDP-Xyl is generated through a transformation of UDP-GlcA inside and outside of the Golgi. Depending on whether the glycosidic linkage is formed via retention or inversion of the stereocenter compared to the donor, GTs can be classified as either inverting or retaining. The GT43 family contains only the inverting kind.³⁰ The mechanism usually involves activation of the phosphate through chelation by some metal ion and subsequent S_N2 substitution on the anomeric center.²⁹

One lesser understood phenomenon is the tetrasaccharide unit Xyl-Rha-GalA-Xyl at the reducing end of xylans in *Arabidopsis*. It has been shown that this

tetrasaccharide plays a very important role in the xylan biosynthesis, although it is not clear how. Two hypotheses have been proposed:¹⁴

1. The tetrasaccharide might act as a primer from which the xylan synthesis begins by elongation at the non-reducing end.
2. The tetrasaccharide might act as a terminator of the xylan chain after a certain length has been reached.

Interestingly, this tetrasaccharide has not been found to occur in grasses and alternative explanations of how the xylan backbone is synthesized in those cases are still lacking. What seems to be clear so far is that several GTs from the GT61 family as well as other enzymes are needed to work interdependently in a xylan synthase complex to synthesize the xylan backbone.¹⁴

According to Pauly,¹⁴ there exists a better understanding of the substitution of xylans. For the attachment of GlcA and Araf to the xylan backbone, various genes have already been identified in *Arabidopsis* as well as wheat and rice. Glucuronic acid has been found to be added to the xylan backbone by glucuronosyltransferases (GUX). It has also been found that the arabinofuranose substituents are generated through a series of transformations from UDP-Xyl to UDP-Arap to UDP-Araf by an epimerase and mutase respectively outside of the Golgi and then somehow transported into the Golgi. Some progress has also been made on identifying the enzymes responsible for the modification of xylans, for instance the O-4 methylation of glucuronic acid residues by GX methyltransferase (GXMT/GMX3) using S-adenosylmethionine or the O2- and O3-acetylation of xylans with AcCoA. During the course of that research it has also been shown that these modifications are essential for the function of xylans. Rennie and Scheller have provided a graphical overview of the current understanding of the xylan synthesis in the Golgi apparatus:¹⁷

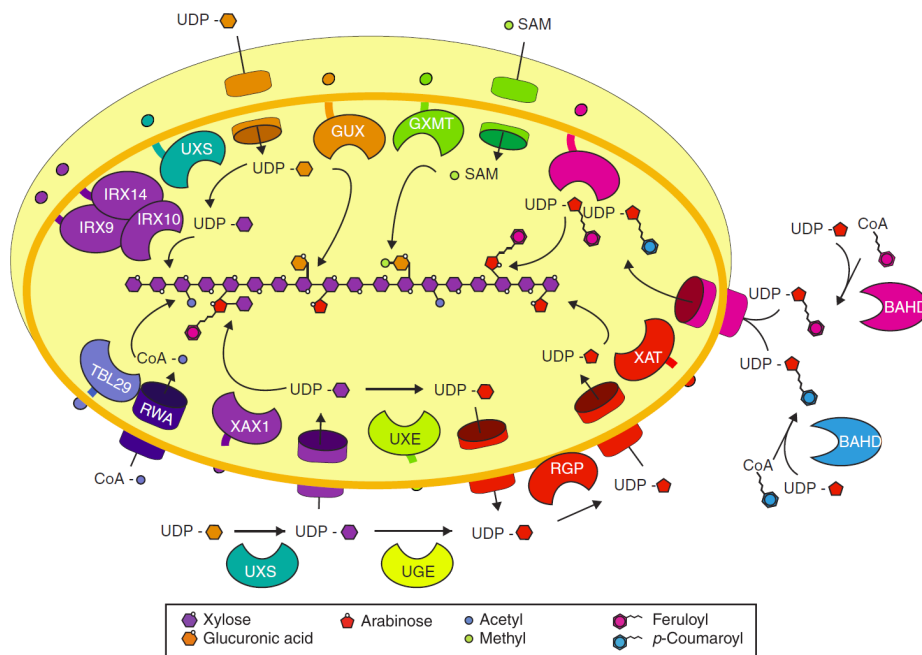


Figure 1.7: An overview of the Xylan biosynthesis in the Golgi apparatus, adapted from Rennie and Scheller.¹⁷

1.3.3 Degradation

On the quest to independence from fossil fuels and petrochemistry, considerable effort is being made to derive energy and valuable chemicals from biomass. The obvious advantage would be the ability to escape the limited supply of oil by using a sustainable process of growth, harvest and recycling while at the same time producing less waste and pollution.³¹

Essential steps towards realizing this vision consist of understanding, modifying and utilizing the natural degradation processes of plant polysaccharides into their monosaccharide units. The natural processes leading to the degradation of plant material are highly efficient, but slow.³²

Hydrolysis of hemicellulose has been shown to be complicated by the fact

that a number of different enzymes are necessary to degrade it. For example, debranching without depolymerization of the backbone has been shown to lead to intermolecular aggregation leading to precipitation which makes further depolymerization impossible.^{33–36} The best way to handle this problem has been found to be the use of different enzymes simultaneously.^{37–40}

The two main groups of hemicellulose degrading enzymes can be divided into main-chain enzymes and side-chain enzymes. The main-chain enzymes include β -1,4-D-xylanases and xylosidases. Side-chain enzymes include α -arabinofuranosidases, acetyl xylan esterases and α -D-glucuronidases (Figure 1.8).^{33,41}

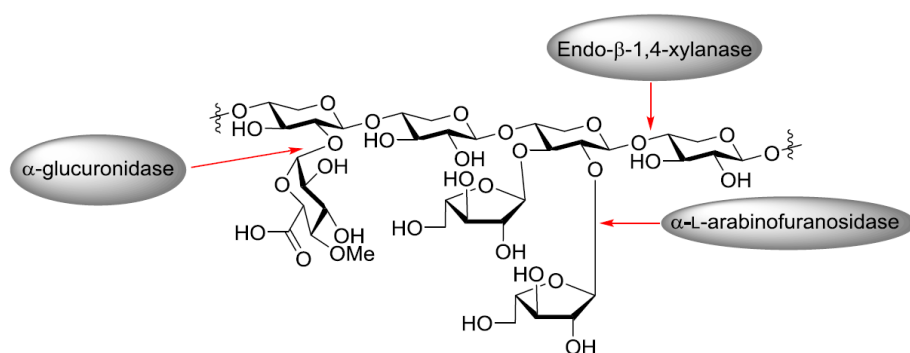


Figure 1.8: Schematic representation of enzymes involved in the hydrolysis of a substituted xylan⁴²

The degradation of linkages between carbohydrates and lignin is facilitated by enzymes such as acetyl xylan esterase, ferulic acid esterase and *p*-coumaric esterases which attack the hemicellulose side chains and break any bonds to lignin.⁴³ The delignification process still needs to be optimized and a better understanding of all the enzymes involved is necessary.

Xylanases

The best studied xylanases belong to glycosyl hydrolase (GH) families 10 and 11.⁴⁴ In order for these enzymes to cleave the 1,4- β -D-xylosidic linkages it is

required for the backbone to have at least three consecutive, unsubstituted xylose units. That makes these enzymes unsuitable for the degradation of more densely substituted xylans. A more suitable enzyme has recently been discovered. This arabinoxylanase from the GH5 family has an extra binding pocket to accommodate an arabinose unit close to the cleave site.⁴⁵ Other examples of xylanases accommodating substituents close to the active site are GH30 glucuronoxylanases.^{46,47} This is significant in light of findings that have shown some xylanases to be inhibited by the presence of glucuronoxylanases.^{48,49} GH30 glucuronoxylanases have been shown to cleave glucuronoxylans by binding 4-OMe-GlcA or unmodified GlcA substituents at the -2 subsite.⁵⁰

Accessory Xylanolytic Enzymes

Those enzymes that remove the main chain substituents in xylans are called accessory xylanolytic enzymes. They can be divided into two groups:⁵¹

- Enzymes that remove side chains only from short branched oligosaccharides generated by endoxylanases
- Enzymes that remove side chains from both, polymeric and oligomeric substrates

It has been shown that GH67 α -glucuronidases act synergistically with xylanases to liberate MeGlcA from glucuronoxylans.^{52,53} The synergistic effect has been demonstrated repeatedly. After the xylanase degrades the backbone, the glucuronidase acts on short oligosaccharide fragments to release those MeGlcA residues that are connected to the non-reducing terminal end of the xylan chain.^{54,55}

A more recently discovered class of α -glucuronidases is GH115.^{56–58} This class differs from GH67 in that it is able to release MeGlcA linked to internal as well as non-reducing terminal xylopyranosyl residues. A recent study has demonstrated that GH115 enzymes act as inverting glycoside hydrolases, releasing MeGlcA as its β -anomer.⁵¹ It has also been shown that substrates can have different

lengths from two to five xylose units. An increase in length led to an increase of substrate specificity indicating that the enzyme binds to the MeGlcA residue and two adjacent xylopyranosyl residues.⁵¹

Among arabinofuranosidases four enzyme families have been identified so far. An extensive overview is given by Lagaert:⁵⁹

- GH43: Some enzymes in this family have been found to cleave arabinose at the O-3 position from xyloses with substitutions at the O-2 as well as the O-3 position.^{60–64} Others exclusively hydrolyze arabinose linkages from xyloses that have only one arabinose residue linked to either the O-2 or O-3 position.^{60,65} The two types can work in synergy when the first one removes the arabinose on the O-3 position, making more substrate available for the second kind.^{60,62,66}
- GH51: Except for four endoglucanases, this family contains only arabinofuranosidases and contains the largest number of studied arabinofuranosidases. Most of the arabinofuranosidases have wide substrate specificity and are able to cleave arabinose from longer as well as shorter chains. Most enzymes show a preference towards cleaving either from the O-2 or O-3 position and studies have shown that the active site contains only enough room for monosubstituted xyloses.^{62,67} Nevertheless, small activity towards disubstituted xyloses has been observed as well.^{68,69}
- GH54: Most enzymes found in this family show selective activity towards arabinose bound to the O-3 position on singly substituted xylose residues at the non-reducing end of arabinoxylan oligosaccharides. Activity towards the middle xylose in xylotriose has been much reduced or absent.^{70–72}
- GH62: This family contains exclusively arabinofuranosidases and all enzymes release arabinose from arabinoxylans.^{68,73–77} Until now three enzymes have been studied and all have shown to cleave arabinose from a monosubstituted xylose and never from a disubstituted moiety. Two of the enzymes showed a preference towards cleaving from the O-3 position over against the O-2 position.^{68,75}

The continued study of hemicellulose degrading enzymes will be much aided by

a higher availability of pure, well-defined oligosaccharides. This is especially the case for the study of those enzymes that depend on the prior cleavage of the substrate into smaller units. Currently this availability is relatively low and most studies rely on impure polysaccharides and their mixed degradation products. Chemical synthesis of relevant enzyme substrates can therefore prove to be very valuable to the biological and biochemical community in this area of modern research.

1.4 Chemical Synthesis of Oligosaccharides

The most exhaustive overview on carbohydrate chemistry in general and oligosaccharide synthesis in particular can be found in Fraser-Reid's 3000 page reference work *Glycoscience*.⁷⁸ Additionally Demchenko's *Handbook of Glycosylation Chemistry* gives a detailed overview over all modern glycosylation methods.⁷⁹

The synthesis of oligosaccharides is marked by a constant focus on chemoselectivity, stereoselectivity and reactivity. Two factors are especially characteristic of oligosaccharide synthesis:

1. On the one hand, the multitude of similar functional groups make it difficult to connect two carbohydrate molecules in a specific way.
2. On the other hand, reactivity between different carbohydrates can differ in unexpected ways, often times necessitating a lot of trouble shooting and adjustments in one's chosen synthetic strategy.

Despite these difficulties, modern chemical synthetic methods are capable of overcoming most of these problems, given enough time and resources. A multitude of protecting groups can be used today to overcome the problem of the similarity of functional groups. The problem of reactivity has been and still is being addressed by the introduction of a variety of glycosylation methods of which one can usually expect at least some to work well for a particular problem. In addition to these advances, we have today a better understanding of the relationship between utilized protecting groups and reactivity as well as chemoselectivity than ever before. A short survey of the most successful strategies to overcome the typical problems shall be outlined here.

1.4.1 Mechanistic Considerations

The Glycosyl Cation

In order for a new glycosidic bond to be formed, the old one needs to be broken. The question that has prompted intense investigations concerns the mechanism of the bond breaking. Is there a glycosyl cation? If so, does it exist as a contact ion pair or a solvent separated ion pair? Does the glycosylation reaction follow a S_N1 or S_N2 pathway? How much do protecting groups and solvent influence the answers to these questions? A review by Bohe and Crich in 2014 gives an overview of the research done on this topic.⁸⁰ In general the question of whether a S_N1 or S_N2 pathway is followed needs to be answered for every individual reaction and many times an in-between mechanism is assumed.⁸¹ A number of computational and indirect methods have been employed, trying to prove the existence of the glycosyl cation. Bohe and Crich concluded that the evidence points towards the existence of these cations in some cases. Very recently, glycosyl cations have been directly observed in low temperature NMR experiments.⁸² The cations were formed by adding different sugar derivatives to a solution of HF/SbF₅. In this superacidic⁸³ medium all acetate protecting groups are protonated and the anomeric acetate reacts further to give the corresponding glycosyl cation (Figure 1.9).

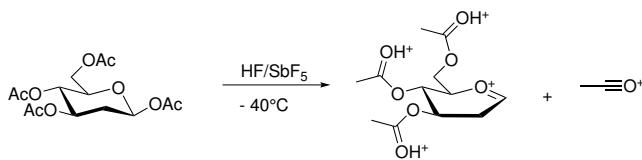


Figure 1.9: In a superacidic solution the glycosyl cation obtained from peracetylated 2-deoxyglucose is stable and observable by NMR at -40°C.⁸²

The experiment was conducted with peracetylated 2-deoxyglucose as well as with 2-bromoglucose. In both cases the cation has been observed. The NMR data showed a ¹H-NMR shift for the anomeric proton of the 2-deoxy sugar at 8.89 ppm and a ¹³C-NMR shift of 229.1 ppm. The 2-bromo derivative has a ¹H-NMR

shift at 8.36 ppm and a ^{13}C -NMR shift of 198.1 ppm. The chemical shifts and coupling constants in combination with calculations showed that the 2-deoxy derivative assumes a 4E conformation whereas the 2-bromo derivative assumes a 4H_5 conformation in which the bromine coordinates towards the anomeric carbon. This difference in conformation led to a difference in products when both cations were deuterated. Deuteration with cyclohexane- d_{12} ^{84,85} led to the formation of mostly the deuterated α -product for the 2-deoxy sugar and mostly the β -product for the 2-bromo sugar. Both results are in accordance with the presumed conformations.

The Anomeric Effect

Any glycosylation reaction can in theory have two stereochemical outcomes. Typically, the glycosyl donor needs to be activated by a promoter. Activation leads to the formation of a glycosyl oxocarbenium ion,⁸² which can then be attacked from either side by an acceptor. The product can be the α - or the β -anomer (Figure 1.10).

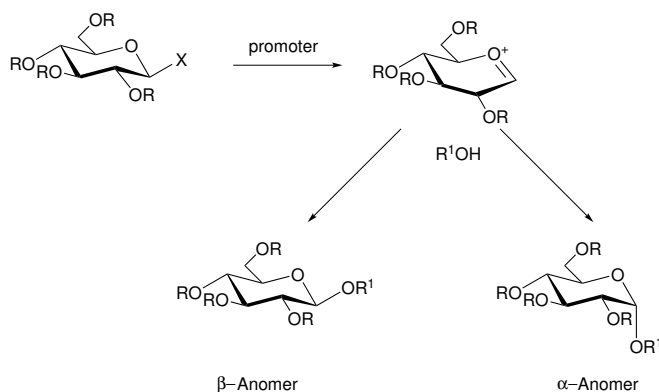


Figure 1.10: The two possible stereochemical outcomes of a glycosylation, exemplified on a glucose derivative.

It has been observed that glycosylation reactions under thermodynamic conditions generally lead to the α -anomer.⁸⁶ Two different explanations have been given for that phenomenon. The first one is based on favourable dipole-dipole in-

teractions for the alpha anomer, while the second one proposes that the α -anomer is stabilized by the $n(\text{O}) \rightarrow \sigma^*(\text{C-X})$ orbital interaction. The debate surrounding which explanation would be the correct one is ongoing. An argument in favour of the electrostatic model has been made in 2010 based on calculations.⁸⁷ Often times it is argued that α -selectivities in modern glycosylation methods are due to the anomeric effect. Ian Cumpstey refuted this idea in 2012, arguing that the anomeric effect can not account for selectivities in glycosylation reactions under kinetic control. It is rather the structure of the individual substrate, the particular reaction conditions and ultimately the transition state energies that determine the stereoselectivities.⁸¹

Participating Protecting Groups

The most frequently employed tool to perform glycosylations with high stereoselectivities is the use of ester protecting groups on C-2. This was described by Lemieux in 1954.⁸⁸ Participation of these groups during glycosylation reactions leads to a blocking of the anomeric center from the side of the protecting group, forcing the acceptor to attack from the other end (Figure 1.11). This leads usually to the exclusive formation of 1,2-trans-glycosides. The same study that proved the existence of glycosyl cations mentioned above, managed to also prove the existence of the dioxazolinium cation by NMR.⁸²

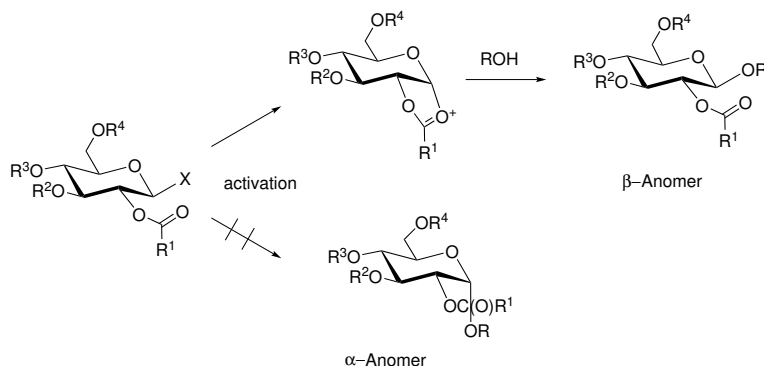


Figure 1.11: A carbonyl-containing protecting group on the 2-position can participate in the reaction by blocking the anomeric center from one side, thereby leaving the incoming acceptor with the only option to attack from the opposite side.

Armed/Disarmed Effect

In order to connect two carbohydrates in a specific way, the most common method is to block all functional groups except for the one that is desired to be connected. In carbohydrate chemistry, protecting groups play more roles than just blocking off reactive functional groups. They have been found to be crucial in tuning chemoselectivity as well as stereoselectivity of glycosylation reactions. An exhaustive overview of this topic can be found in Fraser-Reid's 2011 book *Reactivity Tuning in Oligosaccharide Synthesis*.⁸⁹ An overview over the most common protecting groups and their usage can be gained from the chapter on protecting groups in *Comprehensive Glycoscience*.⁹⁰

The phenomenon of the armed/disarmed effect describes the correlation between certain kinds of protecting groups on a glycosyl donor and its reactivity. This has been mentioned by Paulsen in 1982⁹¹ and formalized and given its name by Fraser-Reid in 1988.^{92–94} Donors containing ester groups are generally less reactive than those containing ether groups since the electron withdrawing effect of the ester carbonyl destabilizes the potential glycosyl cation. This insight allows one to adjust the reactivity of the donor depending on the individual problem. These reactivity differences have been famously exploited by Wong's

research group when they synthesized a library of 50 different thioglycosides to construct a relative reactivity scale.^{95–97} This reactivity scale allowed them to perform computer optimized one-pot procedures toward oligosaccharides in which the thioglycosides would be activated one after another according to their reactivity (Figure 1.12).

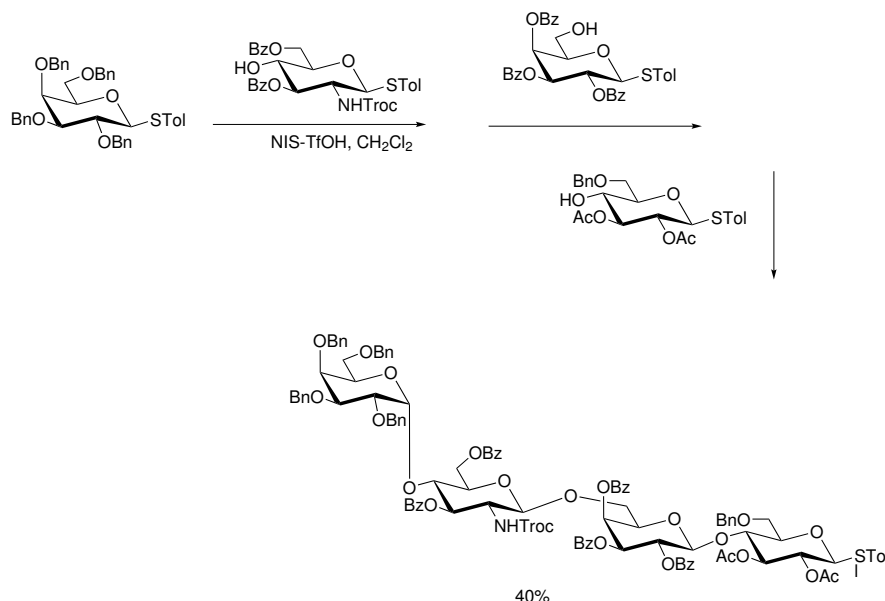


Figure 1.12: One-pot synthesis of a tetrasaccharide based on the reactivity differences between donors and acceptors, depending on the armed/disarmed effect.⁹⁵

In more recent years the concept of super-armed and super-disarmed donors has been introduced by Bols et al.^{98,99} Unlike the previous donors, these donors derive their reactivity not from their electronic but from their steric properties. A detailed account of the development of this field can be found in *Reactivity Tuning in Oligosaccharide Synthesis*⁸⁹ and specifically in a whole chapter on the topic of superarmed and superdisarmed donors.⁹⁴

1.4.2 Glycosidic Bond Formation

Glycosidic bond formations generally proceed according to the same general principle. The heteroatom attached to the anomeric center undergoes electrophilic attack which leads to the formation of a glycosyl cation which in turn is able to react with the glycosyl acceptor. Depending on the circumstances of the reaction the solvent, protecting groups and the overall electronic structure of the molecule, intermediates undergo more or less something between an S_N1 and S_N2 reaction.

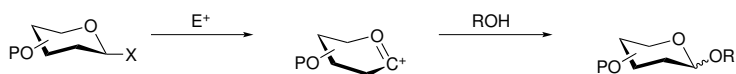


Figure 1.13: General principle of glycosidic bond formation

Fischer Glycosylation

Emil Fischer was the first to describe the general principle of glycosylating alcohols with sugars in 1893.¹⁰⁰ He showed that in principle any alcohol can react with any sugar when dissolved in a solution of hydrochloric acid in the respective alcohol to form the corresponding glycoside.

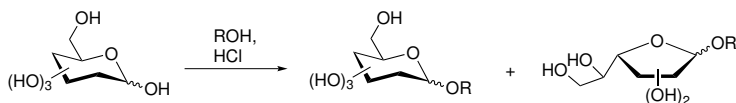


Figure 1.14: Under Fischer glycosylation conditions, one can get either the furanose or pyranose form, and either the alpha or beta mixture. Because of the nature of the chemical equilibrium, over time the most stable product will be dominant in the reaction mixture.

This method is still used today for the synthesis of simple aliphatic glycosides. Its disadvantages are the formation of several byproducts and harsh reaction conditions. A modern improvement has been found by Izumi et al. in 2002.¹⁰¹ They describe a procedure in which hydrochloric acid is generated in situ by using TMSCl at room temperature. This allows for the formation of propargyl

glycosides, which cannot be formed under standard Fischer conditions due to polymerization.

Koenigs-Knorr Glycosylation

A major milestone in glycosylation reactions has been the development of glycosyl bromides by Wilhelm Koenigs and Eduard Knorr in 1901.¹⁰² Glycosyl bromides were originally synthesized from fully acetylated carbohydrates. The glycosyl bromides were then activated through silver carbonate, allowing for an attack of a nucleophile to perform the glycosylation. Later developments showed that this procedure can also be applied to glycosyl chlorides and iodides.¹⁰³ Helferich popularized the use of mercury salts as activating agents¹⁰⁴ and a variety of silver and mercury salts have been used.¹⁰⁵ Hydrolysis can be easily prevented by using molecular sieves. An alternative way of synthesizing the glycosyl halides is to generate them from thioglycosides.^{106,107} Variations of the Koenigs-Knorr method have been the main backbone of oligosaccharide synthesis until the development of more advanced methods in the 1970s and 1980s.^{108–110} The most prominent development has been the introduction of glycosyl fluorides.¹¹¹ Their high stability led to a neglect by the scientific community until it was discovered that they can be activated by many promoter systems, for example $\text{SnCl}_2\text{-AgClO}_4$.¹¹² The high stability, ease of synthesis and a mild promoter system makes glycosyl fluorides a useful tool in oligosaccharide synthesis.¹¹³

Thioglycosides as Donors

Thioglycosides were first synthesized by Fischer and Delbrück in 1909.¹¹⁴ They observed that these compounds are significantly more stable towards hydrolysis than their oxygen counterparts. Bamford et al. did kinetic studies to analyse the mechanism of the hydrolysis, but did not attempt to utilize them in any synthetically useful way.¹¹⁵

Thioglycosides were publicly presented as a new donor class in 1973 by Ferrier et al. in an article with the prescient title *A Potentially Versatile Synthesis of*

Glycosides.¹¹⁶ This class would indeed become one of, if not *the* most versatile one. Ferrier et al. used phenyl thioglycosides as donors and mercury salts as activating agents for glycosylation reactions.

While Fischer originally prepared his thioglycosides by treating the peracetylated glycosyl bromide with the thiol and sodium hydroxide,¹¹⁴ nowadays thioglycosides are most commonly prepared by treating peracetylated carbohydrates with the thiol under Lewis-acidic conditions¹¹⁷ as has been originally established by Lemieux in 1951.¹¹⁸ The most popular Lewis-acids for this purpose are $\text{BF}_3 \cdot \text{Et}_2\text{O}$ ^{119–123} and SnCl_4 ,¹²⁴ although many more have been described.^{117,119,125,126}

One of the strengths of this donor class is, that it can be converted into any of the other donors (Figure 1.15). This is an advantage, if it turns out that the thioglycoside is not able to perform the desired glycosylation. The treatment with molecular bromine has been described already in 1958 by Weygand.¹²⁷ Alternatively iodonium bromide can be used as well.¹²⁸ Glycosyl fluorides are accessible via NBS activation and reaction with DAST.¹²⁹ Access to trichloroacetimidates is available indirectly through simple hydrolysis with NBS in aqueous acetone and similar systems.^{130,131} Glycosyl sulfoxides can be obtained by oxidation with *m*CPBA.^{132,133}

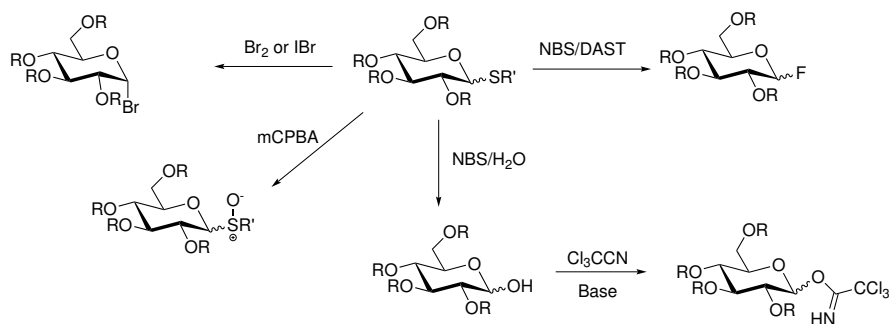


Figure 1.15: Thioglycosides can be converted into any of the other major donor classes.¹¹⁷

As mentioned before, since 1977 thioglycosides have been primarily used not as a bridge towards a variety of glycosyl donors (although a useful property), but

as a donor itself. Ferrier initially used mercury(II)acetate as a promotor. Other heavy metal salts have been used, but generally gave poor yields, which hindered the general adoption of these donors. In 1985 Hans Lönn showed that methyl triflate can act as a promotor.^{125,134} The yields achieved with this method were generally good and heavy metals could be avoided altogether. The disadvantage of using methyl triflate lies in its high toxicity which is caused by its extremely strong ability to methylate. This ability can also lead to methylation of the acceptor.¹¹⁷

Nowadays there are a lot of options for activation, which have better properties. DMTST as a promotor was described by Fügedi and Garegg a year later.¹³⁵ It's less reactive, but still quite sensitive to moisture and requires careful storage under dry, cold conditions. Some of the more popular choices today are NIS/TfOH or NIS/TfOTMS,¹³⁶ BSP/Tf₂O,¹³⁷ diphenylsulfoxide/Tf₂O,¹³⁸ phenylsulfenyl chloride/AgOTf¹³⁹ and its derivative *p*-NO₂PhSCl/AgOTf.¹⁴⁰ The respective advantages and disadvantages usually relate to the temperature at which the donor can be activated as well as the stability of the promotor. For example, NIS is usually used at around -30°C, whereas phenylsulfenyl chloride activates donors at -78°C. On the other hand phenylsulfenyl chloride is not shelf-stable, but its 4-nitro derivative is stable over several weeks when stored at 4°C. A recent review lists 66 different promoter systems.¹⁴¹

The reactivity of thioglycosides can partly be tuned by choosing the substituent on the sulfur. A study by Oscarson and Lahmann showed that the reactivity is dependent on the ability of the substituent to withdraw or donate electron density.¹⁴² Since activation proceeds via electrophilic activation one would expect electron withdrawing substituents to reduce the reactivity whereas electron donating substituents to increase it. This is in accordance with what has been observed.¹⁴² The reactivity scale established in that study is as follows:



Halide substituted phenyl groups have been found to be inert under the reaction with DMTST, but could be activated with NIS/AgOTf. These differences though are not enough to activate a donor in the presence of an acceptor differing only in the sulfur substituent.¹⁴²

Another aspect of thioglycoside chemistry is the possibility to preactivate them in the absence of an acceptor.¹⁴³ By first generating a glycosyl triflate, which can be stabilized at low temperatures, one can ensure full activation of a donor before adding another thioglycoside as an acceptor. This has been exploited in sequential one-pot procedures.^{144–148} For example, in 2013 Gao and Guo presented their synthesis of a heptasaccharide lipomannan in which a major building block was synthesized in a one-pot fashion based on a pre-activation procedure (Figure 1.16).¹⁴⁸ The obtained tetrasaccharide was afterwards transformed into a glycosyl acetimidate to be coupled to another trisaccharide.

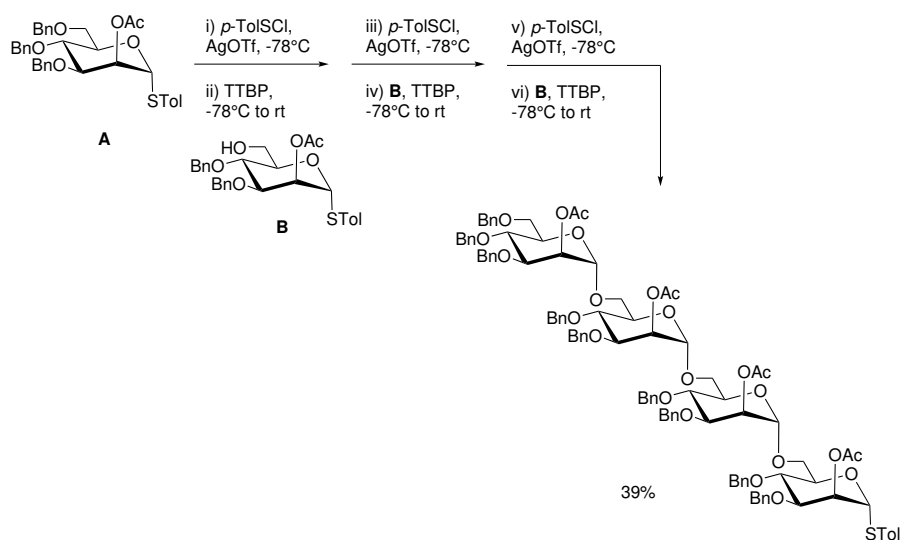


Figure 1.16: One-pot sequential glycosylation towards a tetrasaccharide in which the donor was preactivated with *p*-TolSCL before addition of the acceptor. The product of the coupling could in turn be activated and coupled in the same way until the tetramer was reached in an overall yield of 39%.¹⁴⁸

A review by Cai et al. in 2014 gives an overview of how sulfenyl triflates have been used to generate glycosyl triflates and summarizes mechanistic studies on the topic.¹⁴⁹

The first observation of glycosyl triflates by NMR has been made by Crich and Sun in 1997.¹⁵⁰ They generated the triflates by treating the corresponding

glycosyl sulfoxides with triflic anhydride at -78°C . Among other things they found out that the sulfenyl triflate is so reactive that it immediately generates the glycosyl triflate and cannot be detected by itself by NMR (Figure 1.17). They have also studied the stability and selectivity of glycosyl triflates, finding that the results can vary depending on the protecting groups and solvent employed. (Decomposition has been detected between -30°C and -10°C .) Likewise, the efficiency of the glycosylation is still dependent on the individual properties of the donor. Huang et al. found that unreactive donors can be tuned towards giving better glycosylation yields by introducing arming protecting do not groups.¹⁵¹ Contrary to their expectations the arming protecting groups do not facilitate the activation step. Instead the glycosylation step by itself is facilitated.¹⁵¹

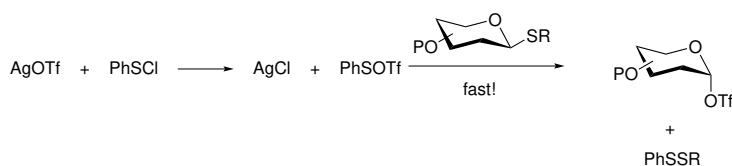


Figure 1.17: The generation of glycosyl triflates from phenylsulfenyl triflates has been found to be so fast that the phenylsulfenyl triflate can only be detected by NMR when generated in the absence of a glycosyl donor.¹⁵⁰

In 1998 Crich et al. reported the application of their insights to a β -mannosylation protocol.¹⁵² They generated a mannosyl triflate by treating a mannosylsulfoxide with triflic anhydride at -78°C . The generation of the α -triflate enabled them to perform several β -mannosylations with high selectivities.¹⁵²

The most important side reaction for thioglycosides is the aglycon transfer. It is possible for acceptors to transfer their thio-aglycon to the donor instead of attacking with their free hydroxyl group. This depends on the reaction conditions and can in part be mitigated by using sterically more hindered thiols.^{153–155}

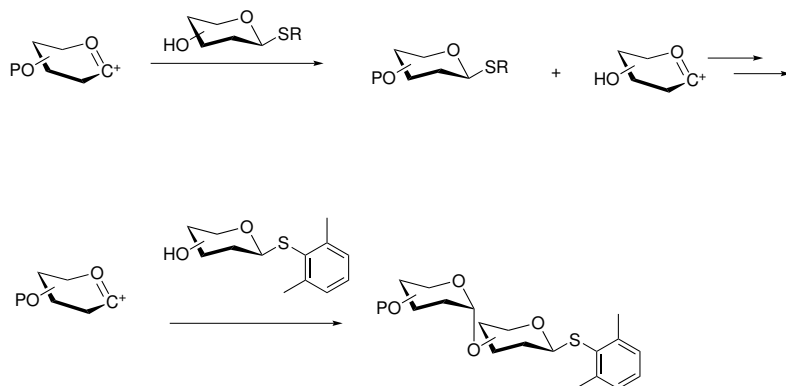


Figure 1.18: Aglycon transfer is a typical side reaction for thioglycoside acceptors. One way to mitigate it is the introduction of more sterically hindered substituents on sulfur.¹⁵⁴

Acetimides as Donors

Acetimides as glycosyl donors were first reported by Pougny et al. in 1977.¹⁵⁶ They reported that glycosyl chlorides can be converted to the corresponding imidate by reaction with silver oxide, base and *N*-methylacetamide. The activation of those imidates was accomplished using PTSA and di- as well as trisaccharides were synthesized in high yields. In 1980 Schmidt and Michel built on those results by introducing glycosyl trichloroacetimides and aryl substituted acetimides.¹⁵⁷ Different from the previous methods, these imidates were not synthesized from glycosyl halides, but from the hemiacetals, circumventing the need for activation with heavy metals (Figure 1.19).

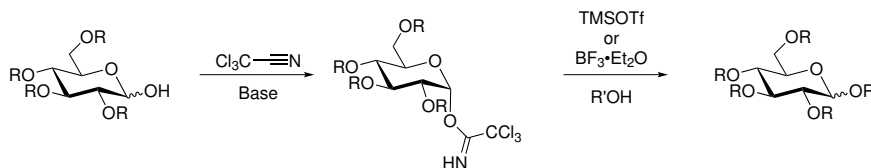


Figure 1.19: Synthesis of trichloroacetimidates can be accomplished starting from the hemiacetal. The base can influence the stereoselectivity of the reaction. The imidate is typically activated with either TMSOTf or $\text{BF}_3 \cdot \text{Et}_2\text{O}$. Typical bases are: DBU, Cs_2CO_3 , K_2CO_3 , NaH

The trichloroacetimidates could be activated with catalytic amounts of boron trifluoride giving the glycosylation products in good yields and good stereoselectivities. The imidates having an α -configuration led consistently to the β -product, independent of the presence of participating groups. The advantages of this method compared to the old one were easier preparation as well as higher reactivity of the imidates.¹⁵⁸ Since then the method has been applied to a wide variety of problems, especially glycoconjugate synthesis.^{159–163}

Although trichloroacetimidates work well in many reactions, there are cases in which glycosylations have been unsuccessful.¹⁶⁴ To solve this problem *N*-phenyltrifluoroacetimidates have been found to provide a solution in many cases. Their synthesis is just as easy (Figure 1.20).

Trifluoroacetimidates have again been first synthesized by Schmidt et al. in 1984.¹⁶⁵ Because of their lower reactivity they have not been studied further until 2001 when Tao and Yu reported the synthesis and application of the *N*-phenyl derivative.¹⁶⁶ They showed how these donors can be used to synthesize oligosaccharides and saponins. They are generally more stable which might help with certain generally unstable donors and has been an advantage in solid-phase synthesis of oligosaccharides.¹⁶⁷ Unlike its predecessor, the formation of this donor is irreversible leading to a mixture of anomers and allowing for the preparation of ketosyl donors.^{168–170} Another area in which the *N*-phenyltrifluoroacetimidates have an advantage is the glycosylation with amides and hydroxamic acids in which the leaving group becomes a competitor with the amide that is to be coupled.¹⁷¹

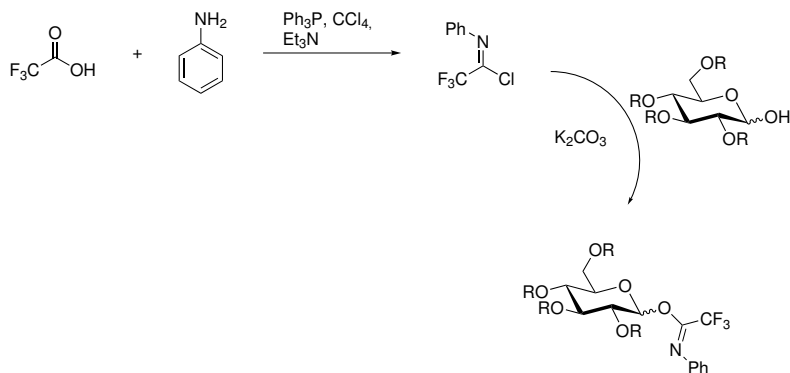


Figure 1.20: The *N*-phenyltrifluoroacetimidoyl chloride is commercially available and can be synthesized in a one-pot reaction combining amide formation and an Appel reaction.^{172,173} The glycosyl imidate is formed analogous to the trichloroacetimidate.

An example of a successful oligosaccharide synthesis based on the acetimidate method is the synthesis of glycoconjugate vaccines by Wu and Bundle in 2005 (Figure 1.21).¹⁷⁴ To reach an oligomannan target they coupled an acetimidate donor to a mannose acceptor. The non-reducing end of the disaccharide was then deprotected at the 2-position and through oxidation and stereoselective reduction the glucose unit was transformed into a new mannose acceptor which in turn could be coupled with an acetimidate donor again. This circumvents the need for a reliable β -mannosylation method by relying on the participating group of the glucose donor. Repeating this procedure another time accomplished the synthesis of the β -(1 \rightarrow 4)-linked tetramannan using only acetimidates in the glycosylation step.

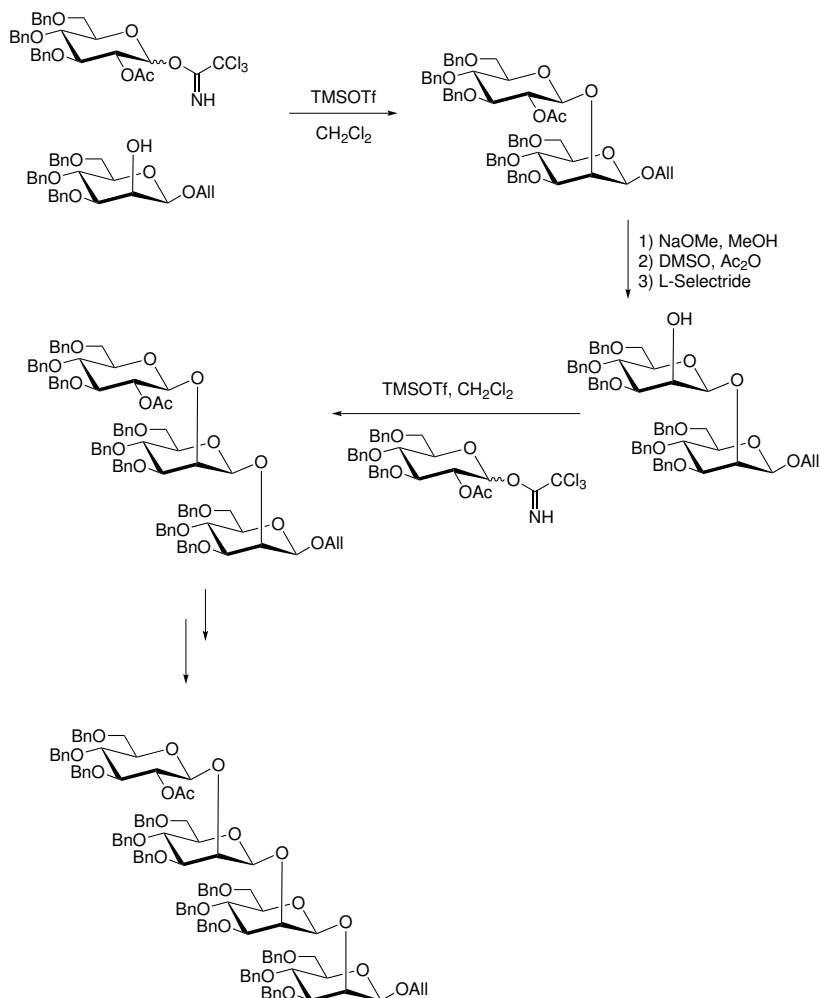


Figure 1.21: Synthesis of a tetramannan by Wu and Bundle¹⁷⁴

1.4.3 Synthesis of Xylans

Xylans can be extracted from plant material. The disadvantage is that only inseparable mixtures can be isolated.^{175,176} For example a recent publication describes the isolation of "well-defined" oligosaccharides by hydrolysis of beechwood xylans with sulfuric acid.¹⁷⁷ Nevertheless, what was obtained were mixtures of

oligosaccharides of different lengths. Generally, isolation of hemicellulose fragments requires careful hydrolysis and chromatography and often time delivers only small quantities of material.^{178–183} This complicates the analysis of enzyme activity and selectivity and so far only chemical synthesis is able to deliver pure, well-defined substrates in higher quantities.

Xylobiose was first synthesized in 1961 by coupling a peracetylated xylopyranosyl bromide donor with a 1,2,3-benzylated xylopyranose acceptor promoted by $\text{Hg}(\text{CN})_2$.¹⁸⁴ Over the next decade, some improvements have been made.¹⁸⁵ Longer β -(1 \rightarrow 4)-linked xylan chains of three to four, five and six units have been synthesized in the early 1980s in a series of publications based on the same Koenigs-Knorr coupling conditions reported by Kovac et al.^{186–188} The couplings have been performed towards the direction of the non-reducing end of the chain by debenzylating the 4-position of the oligosaccharides. At the same time some effort has been made to synthesize branched oligoxylans and glucuronoxylans.^{189,190} The glucuronic acid has been introduced by using the glucuronyl chloride as a donor and silver perchlorate as a promoter to give a separable α/β -mixture (2.7 : 1). Oscarson and Svahnberg synthesized small glucuronoxylan fragments based on xylobiose in 2001 (Figure 1.22).¹⁹¹ Using DMTST in diethyl ether they were able to couple the glucuronic acid donor with complete α -selectivity to the disaccharide acceptor.

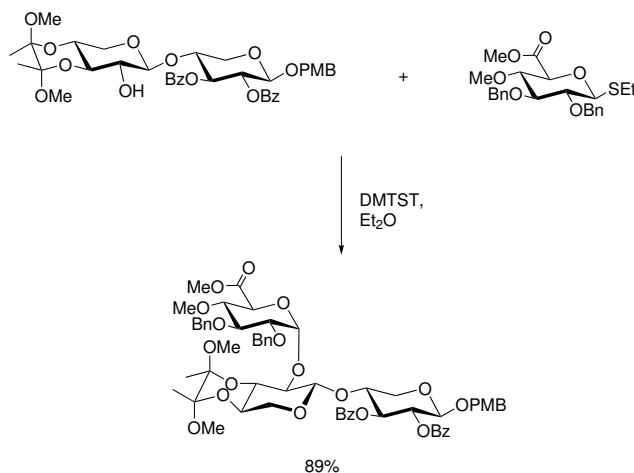


Figure 1.22: Synthesis of a small glucuronoxylan fragment by Oscarson and Svahnberg¹⁹¹ research

In the 1990s, Takeo et al. synthesized a series of oligoxylans of up to four units based on a combination of the Koenigs-Knorr conditions and thioglycoside chemistry, connecting two disaccharides to give a tetrasaccharide.¹⁹² They have afterwards improved their blockwise approach and were able to synthesize oligosaccharides containing ten units of xylose.^{193,194} In 2002 Chen and Kong reported the synthesis of a β -(1 \rightarrow 3)-linked xylohexaose, extending the chain in the direction of the reducing end with a disaccharide building block using acetimidate chemistry.¹⁹⁵ Arabinoxylans have recently been synthesized via solid-phase synthesis by Schmidt et al.¹⁹⁶ Using the automated oligosaccharide synthesizer developed by the group of Peter Seeberger¹⁹⁷ they were able to synthesize β -(1 \rightarrow 4)-linked xylan chains up to the octasaccharide and several shorter arabinoxylan chains carrying up to two arabinose units at the 3-position.

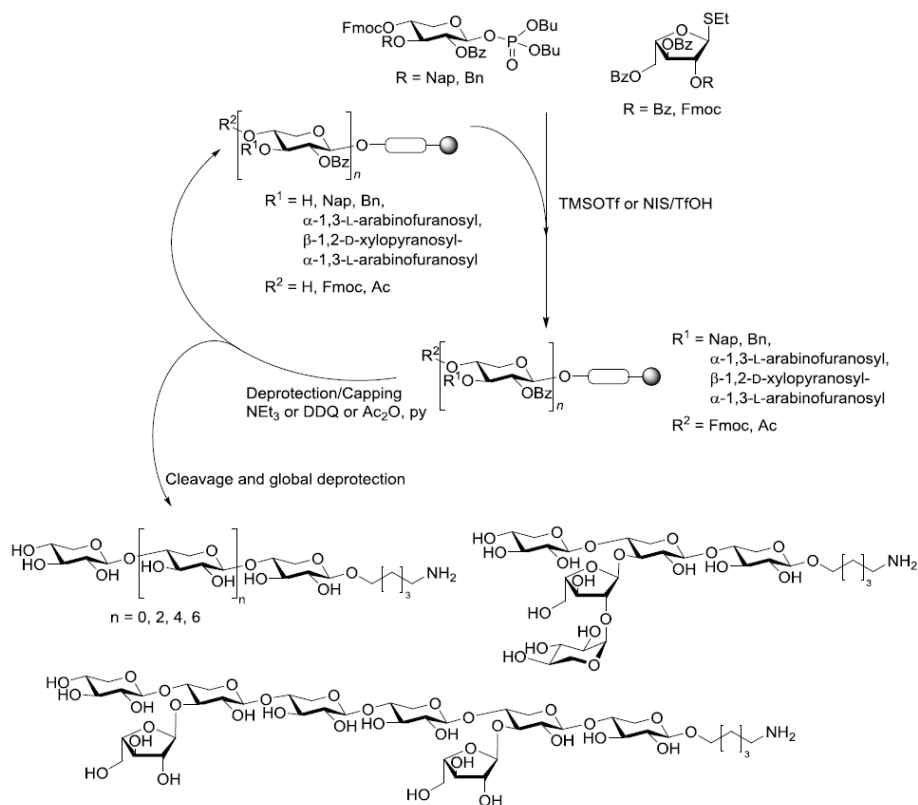


Figure 1.23: Solid phase automated synthesis of some arabinoxylans as described by Seeberger et al.¹⁹⁶

This was made possible by attaching a xylopyranosyl phosphate to a linker attached to the solid phase. By removing the Fmoc protecting group from the 4-position, another unit of a xylopyranosyl phosphate can be added through a TMSOTf promoted glycosylation. The introduction of arabinose to the 3-positions was made possible by selectively introducing benzyl and naphthyl protecting groups. After selective deprotection, the arabinofuranosyl thioglycoside was coupled to the backbone via NIS/TfOH.

1.5 Goal

In order to be able to better study the functions and selectivities of hemicellulose degrading enzymes, the need for higher quantities of a variety of pure, well-defined oligosaccharide substrates needs to be fulfilled. The goal of this project is to develop a reliable synthetic strategy that allows for the construction of various arabinoxylan and glucuronoxylan fragments. The strategy will focus on building up the xylan backbone first. The use of protecting group chemistry will then allow for selective deprotection of either only the O-3 position or the O-2 and O-3 position of selected xylose units in order to attach the desired arabinose or glucuronic acid donors in one glycosylation step to the backbone.

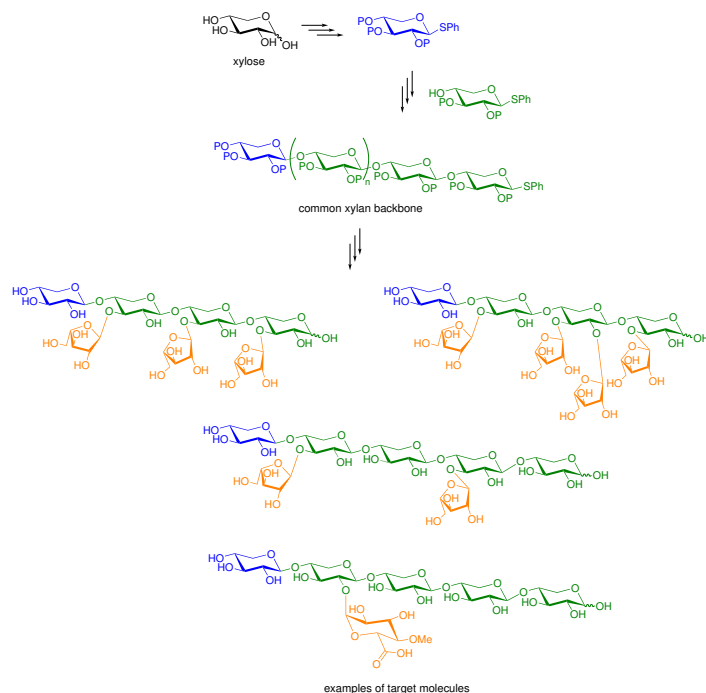


Figure 1.24: General strategy to arrive at arabinoxylan and glucuronoxylan fragments; P = different protecting groups

CHAPTER 2

Results

2.1 Glycosylation with an Unprotected Acceptor

Based on previous work by Agnese Maggi¹⁹⁸ in which a protocol for a regioselective glycosylation with unprotected acceptors based on stannylene acetals has been established, we tried to extend the scope of the reaction towards xylose. This method has so far been only applied to aldohexoses and provided exclusive selectivity for the 6-position.

Retrosynthetically, the problem can be divided into the glycosylation of a perbenzoylated glycosyl bromide and an unprotected thioglycoside (Figure 2.1). There exists literature precedent that xylose can be monoalkylated via tin chemistry on the 4-position.¹⁹⁹

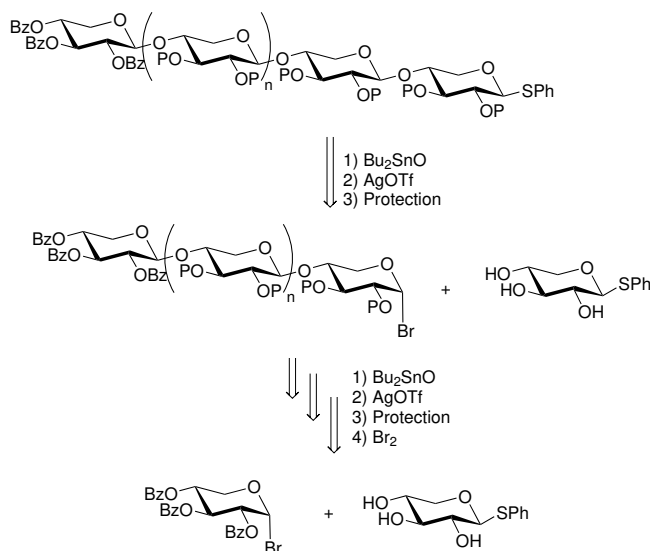
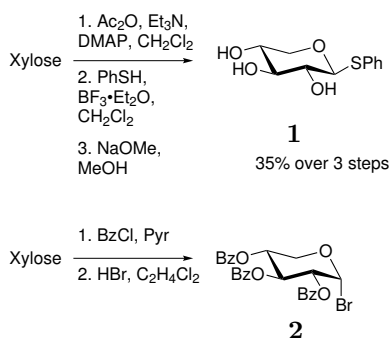


Figure 2.1: Retrosynthetic strategy: Starting from the fully protected bromide and the unprotected thioglycoside, tin-mediated glycosylation leads to the desired 1→4 glycosidic bond. The remaining hydroxyl groups are protected and the thiophenyl group substituted to give the bromide which can undergo the same tin-mediated glycosylation as before.

For that purpose the respective thiophenyl xylose acceptor **1** and xylose donor **2**

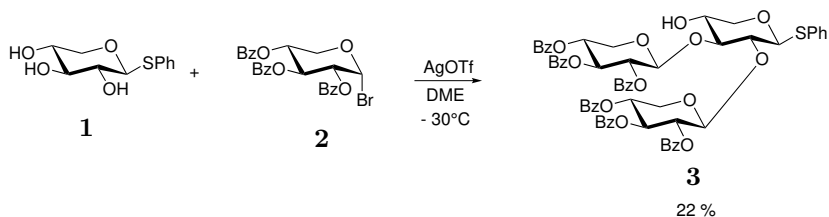
have been synthesized as shown in Scheme 2.1. Xylose is fully acetylated and the thiophenyl group is introduced by the help of a Lewis acid. Deacetylation leads to acceptor **1**. The donor **2** can be obtained by fully benzoylating xylose and treating the product with HBr.



Scheme 2.1: Synthesis of the fully unprotected thioxylloside and perbenzoylated xylosyl bromide.

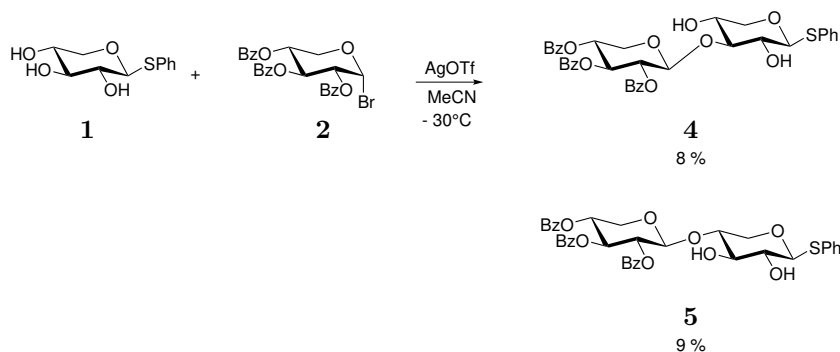
Using the exact same reaction conditions as in the previously established protocol leads to a significant number of unidentified side products. To establish the viability of this reaction, a screening of solvents without any preceding tin acetal formation has been done.

The solvents tested were DMF, DME, DCM, Toluene, MeCN and diethyl ether. Under all conditions yields were low and the formation of a number of side products could be observed by TLC. Generally, the most prevalent product was the trisaccharide **3**. The highest yield for this has been obtained using DME as the solvent (Scheme 2.2). The structure of **3** was determined by NMR. An HMBC experiment shows the attachment of both xylose units to the 2- and 3-positions while the 4-position showed no long-range coupling to either unit.



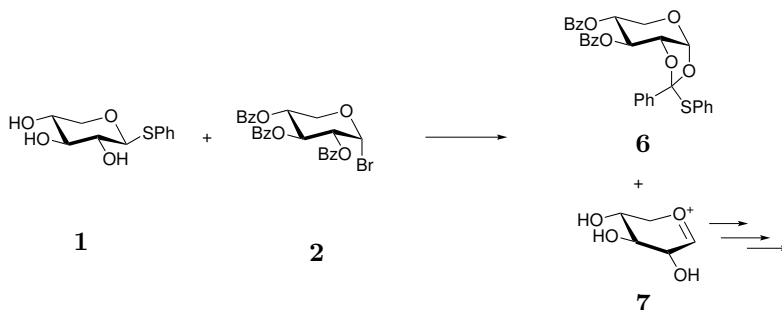
Scheme 2.2: Reaction between the fully unprotected thioxyloside and fully benzoylated xylosyl bromide

Using MeCN, the reaction was slowed down considerably, allowing for the isolation of less than 10% of the undesired regioisomer **4** and the desired one **5**.



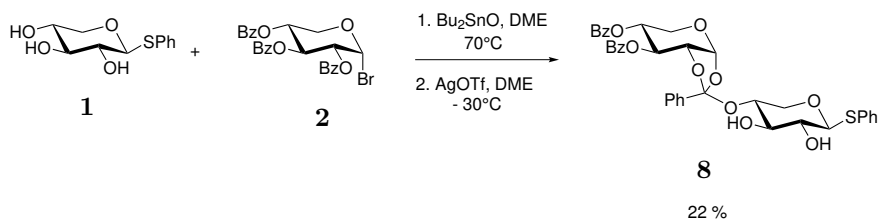
Scheme 2.3: The coupling between acceptor **1** and donor **2** in MeCN leads to the formation of small quantities of the two regioisomeric disaccharides **4** and **5**

In one instance during the screening process, small quantities of the aglycon transfer product **6** have been isolated. This explains the overall poor yields and high amount of side products since this aglycon transfer leads to the formation of the reactive glycosyl cation **7** which is able to react with itself (Scheme 2.4).



Scheme 2.4: Aglycon transfer leads to formation of highly reactive glycosyl cation **7** which in turn is able to react with itself. The aglycon-transfer product **6** has been identified via NMR with the orthothioester signal showing at 110.0 ppm.

Since DME as solvent leads to the cleanest reaction with the highest yield, it was chosen as the solvent for coupling the tin acetal of **1** with donor **2**. It was found that slowly adding the donor to a solution of the tin acetal leads to a cleaner reaction. Additionally, forming the tin acetal in DME instead of methanol likewise leads to better results. Adding 1 equivalent of the donor **2** to the solution via a syringe pump over 5 hours resulted in 22% yield of the product **8** (Scheme 2.5).



Scheme 2.5: Optimized reaction conditions lead to formation of orthoester **8**. NMR analysis showed the orthoester signal at 121.6 ppm as well as long-range coupling with the H-4 of the unprotected unit in an HMBC experiment.

This time the main product is the correct regioisomer and could presumably be easily transformed into the desired disaccharide. Nevertheless, the low yield and

continuous formation of side products indicate that the aglycon transfer cannot be stopped even at lower temperatures.

Considering these results, the synthetic approach needed to be changed.

2.2 Preactivation based glycosylation strategy

The second strategy is based on work by Huang et al.¹⁴⁵ They describe an optimized one-pot strategy in which thioglycosides are preactivated with *p*-tolylsulfonyl chloride and silver triflate to be reacted with another thioglycoside acceptor, which in turn is available for a new round of activation right after the coupling. In this way they were able to build a tetrasaccharide in a fairly short amount of time in 55% yield (Figure 2.2).

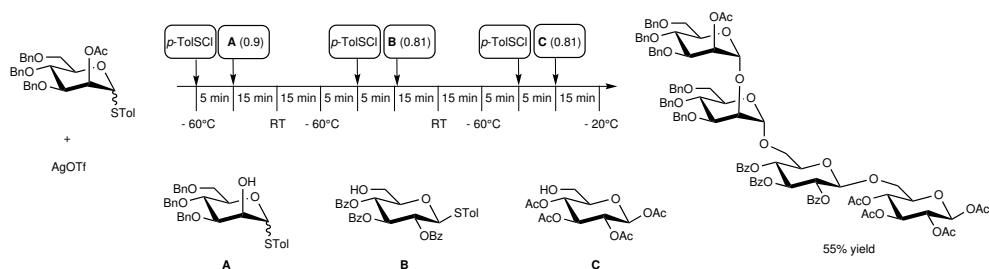


Figure 2.2: One-pot synthesis by Huang et al.¹⁴⁵

The adapted retrosynthetic strategy is depicted in Figure 2.3. This time, the donor is a thioglycoside which can be preactivated. After the activation step a suitably protected acceptor can be added to give the corresponding disaccharide. This disaccharide contains a thiophenyl group at the non-reducing end which can be activated again to be available for another round of glycosylation. This process can then be repeated until the desired chain length is obtained.

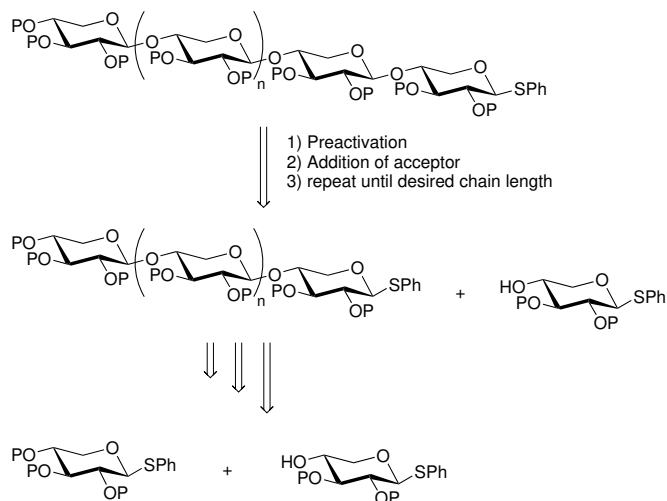
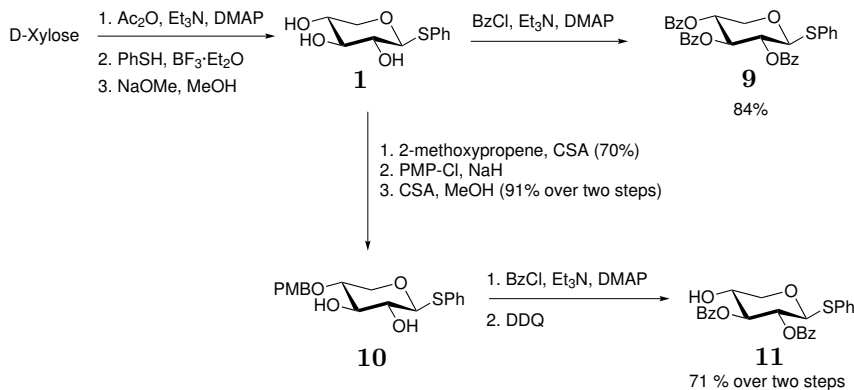


Figure 2.3: Retrosynthetic strategy: A thioglycoside donor is preactivated and subsequently coupled to another thioglycoside acceptor. The new disaccharide contains the thiophenyl group at the reducing end, allowing for another sequence of preactivation and coupling. This process is repeated until the desired length of the backbone is reached. The choice of protecting groups allows for a partial deprotection of the backbone so as to enable a selective introduction of the branching units to the xylan backbone.

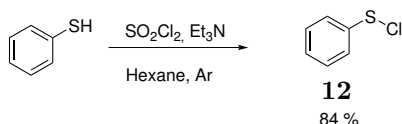
Applying this strategy to the present problem of synthesizing xylans, acceptor and donor were synthesized according to Scheme 2.6. The donor can be obtained by synthesizing triol **1** as described previously and then fully benzoylating it. Synthesis of acceptor **11** is based on a strategy by Yang et al. who worked with *p*-tolyl thioxylosides.²⁰⁰



Scheme 2.6: Synthesis of donor **9** and acceptor **11**.

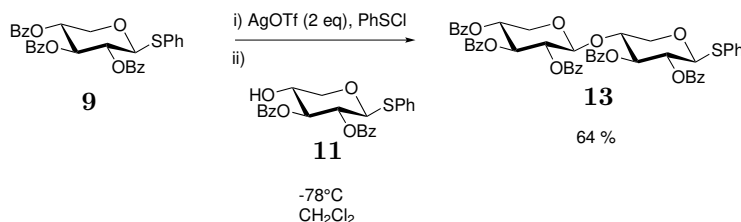
To arrive at the acceptor, the unprotected thioglycoside **1** is first partially protected via acetalization of the 2- and 3-position. This is the most difficult step, mainly because the regioisomers are difficult to separate. The acetal can be formed between the 2- and 3-position or between the 3- and 4-position. Additionally the former regioisomer has been found to undergo further acetalization on the four position with another unit of 2-methoxypropene. Once the desired acetal is isolated, protection of the 4-position with PMB-Cl, cleavage of the acetal, benzylation of the 2- and 3 positions followed by cleaving the PMB-ether leads to acceptor **11**.

Since the promoter is not commercially available due to its instability, it needs to be freshly synthesized according to Scheme 2.7. Thiophenol is reacted with sulfonyl chloride under inert conditions and the sulfonyl chloride subsequently distilled off the reaction mixture under reduced pressure according to a procedure found in a patent.²⁰¹



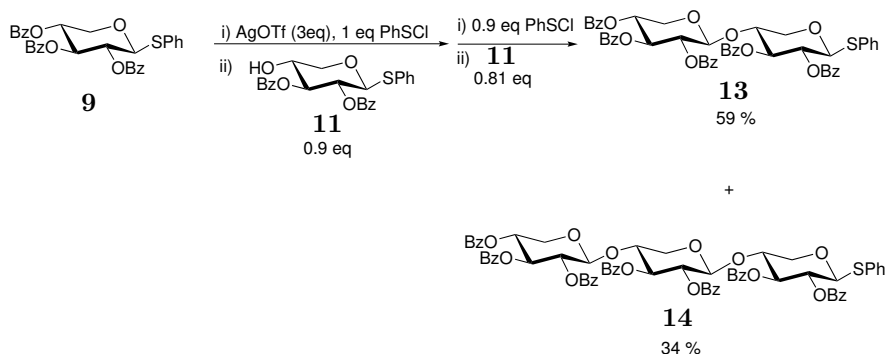
Scheme 2.7: Synthesis of activating reagent phenylsulfenyl chloride.²⁰¹

Applying the same reaction conditions as described by Huang,¹⁴⁵ disaccharide **13** was isolated in 64% yield:



Scheme 2.8: Synthesis of disaccharide **13** employing Huang's reaction condition.¹⁴⁵

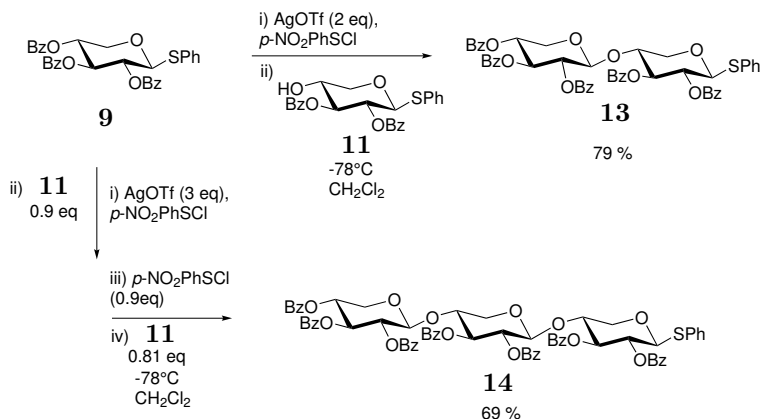
The one-pot protocol towards the synthesis of the trisaccharide **14** leads to mostly disaccharide **13** and a smaller amount of trisaccharide **14**.



Scheme 2.9: One-pot synthesis of trisaccharide **14** leads to the trisaccharide as the minor product next to the disaccharide as the major product.

To investigate whether this can be improved by using a different promoter system a series of experiments has been conducted, testing Me₂S₂/Tf₂O,²⁰² 1-benzenesulfinyl piperidine/Tf₂O²⁰³ and *p*-nitrobenzenesulfonyl chloride/AgOTf.¹⁴⁰ The former two systems led to a number of unidentified side products, and only small amounts of the product as assessed by TLC. Only the last system led to a clean conversion and satisfactory yields of the desired

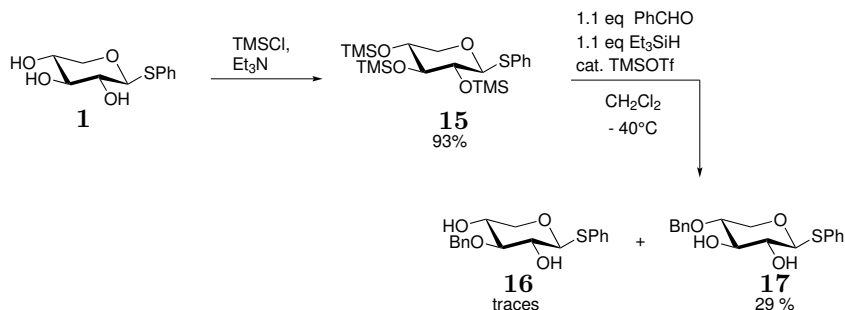
product, surpassing even phenylsulfenyl chloride. With the nitro derivative, the disaccharide could be isolated in 79% yield and the trisaccharide could be isolated in 69% yield after a one-pot procedure if a slight excess of the promoter is used (Scheme 2.10). As has been described by Crich et al.,¹⁴⁰ 1.2 equivalents of the promoter gave the highest yields.



Scheme 2.10: Using *p*-NO₂PhSCl as a promoter, the disaccharide can be obtained in 79% yield. The trisaccharide is obtained in 69% yield when the preactivation strategy is applied as a one-pot procedure.

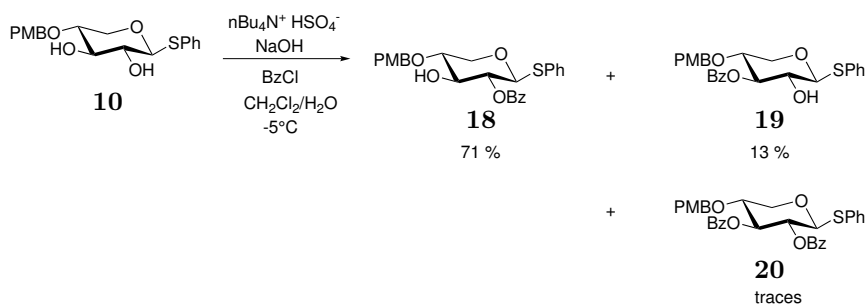
2.2.1 Synthesis of Selectively Protected Acceptors

Encouraged by the previous results, the next step was to find a suitable acceptor containing two different protecting groups so that one arabinose unit can be attached selectively to the xylan chain. According to a procedure by Hung,¹⁴⁷ persilylated phenyl thioxyloside **1** should be selectively benzylated on the 3-position. A subsequent ytterbium triflate catalyzed benzylation was reported to be selective at the 2-position. Unfortunately those results could not be reproduced and only traces of the 3-benzylated product **16** could be isolated next to the 29% of the 4-benzylated product **17** (Scheme 2.11).



Scheme 2.11: The selective benzoylation protocol by Hung¹⁴⁷ could not be reproduced and led to the wrong regioisomer in low yield.

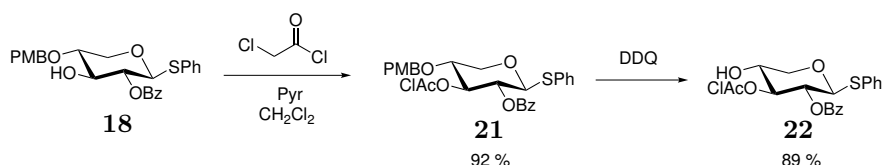
Since the selective benzoylation did not work as expected, different conditions for a selective benzoylation have been tried. Reacting compound **10** with one equivalent of benzoyl chloride in pyridine is slow (12h) and leads to mostly the dibenzoylated product. Benzoylation with silver oxide is likewise similarly slow and leads roughly to a 1:1 ratio between the two possible regioisomers and also some amount of the dibenzoylated product, as could be seen on the TLC. The best way to selectively benzoylate the 2-position is to employ a phase-transfer catalyst. This reaction proceeds to completion within 30-60 minutes, gives high selectivity and a high yield (Scheme 2.12).



Scheme 2.12: Regioselective benzoylation using a phase transfer catalyst leads to compound **18** as the major product.

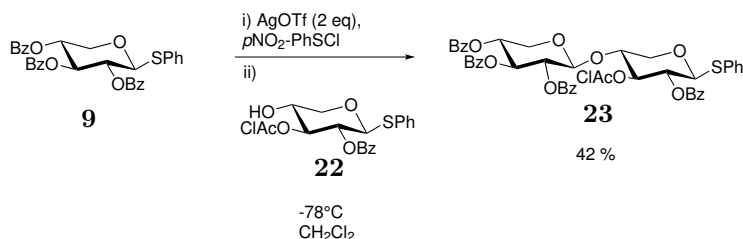
Due to the presence of sulfur, a later deprotection of benzyl groups might be

impossible, even after removal of the thiophenyl group. Therefore only esters were considered as the second protecting group for the acceptor. This has the added benefit of being able to attach it on the 2-position as well, thereby keeping the neighboring-group participation. The introduction of the chloroacetyl group is simple and causes no problems. Subsequent deprotection with DDQ leads to acceptor **22**:



Scheme 2.13: Protection with chloroacetyl.

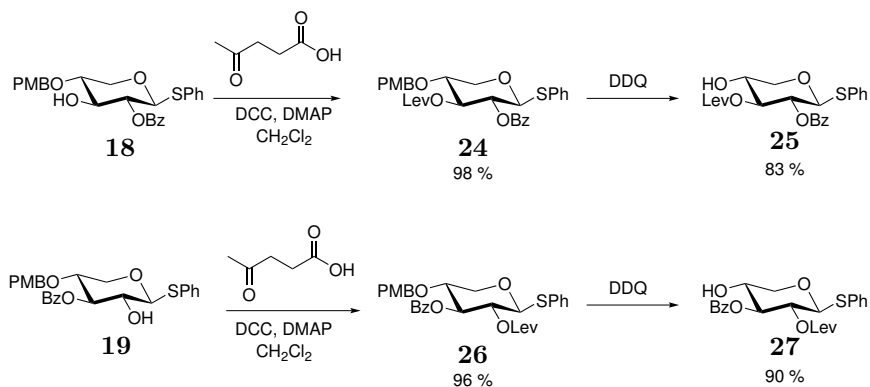
Using the previously established reaction conditions for the glycosylation yields only 42% of disaccharide **23** (Scheme 2.14).



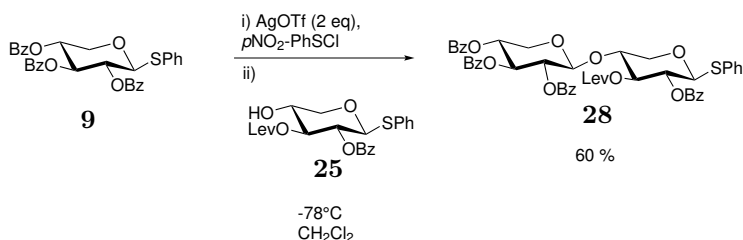
Scheme 2.14: Glycosylation with chloroacetyl protected acceptor **22**

The main reason for this low yield is most likely the lower stability of the chloroacetyl group in the presence of the silver salt.

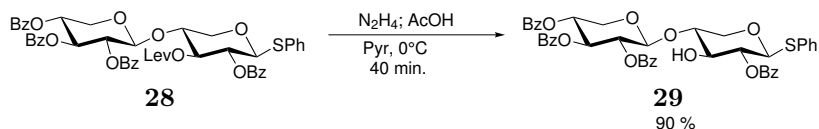
The second ester protecting group that has been tried is the levulinic ester and the synthesis is just as straight-forward as the introduction of the chloroacetyl group. Thioglycoside **18** is esterified using levulinic acid and DCC/DMAP to give the ester **24** in 98% yield. Subsequent deprotection of the PMB ether gives the acceptor **25** in 83% yield (Scheme 2.15).

Scheme 2.15: Synthesis of acceptors **25** and **27**.

Acceptor **25** yielded much better results in the glycosylation, giving disaccharide **28** in 60% yield (Scheme 2.16).

Scheme 2.16: Glycosylation with Lev-group containing acceptor **25**

To test how the deprotection conditions might affect the oligosaccharide, disaccharide **28** was treated with hydrazine. After 20 minutes the starting material was fully consumed. After washing, the deprotected disaccharide **29** was obtained in 90% yield.

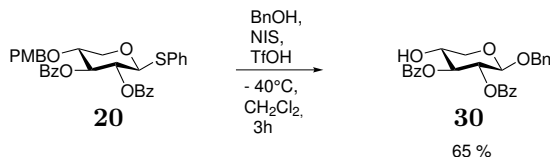


Scheme 2.17: Deprotection of the levulinic ester via hydrazine

Interestingly, the coupling constant of the anomeric proton at the non-reducing end of disaccharides **13** and **28** are relatively low between 4.8 and 4.7 Hz. Lower coupling constants are usually an indication for α -anomers. There are several reasons to assume that in this case the structures are β -anomers with unusual conformations leading to lower tetrahedral angles between the H-1 and H-2 protons which result in lower coupling constants:

- The removal of the Lev group results in a significantly higher coupling constant of 6.9 Hz in compound **29**.
- The CH coupling constants are 184 Hz in both cases, indicating a β configuration.²⁰⁴
- Extension of the chain leads to higher coupling constants in the subsequent units of between 6 and 7 Hz.
- There's no mixture of anomers and the participating effect of the O-2 benzoyl group would strongly favour the β anomer (see section 1.4.1).

The last building block in the pentaxylan precursor of the glucuronoxylan contains a benzyl group at the reducing end. This can be easily synthesized from compound **20** by glycosylation with NIS/TfOH and benzyl alcohol. The reaction is fast and after an extended reaction time, the acid labile PMB group is removed as well, giving acceptor **30** in 65% yield (Scheme 2.18).



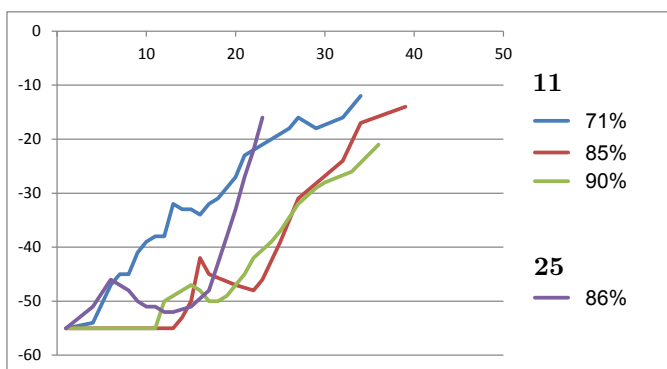
Scheme 2.18: Synthesis of acceptor **30** by glycosylation with NIS/TfOH and benzyl alcohol.

2.2.2 Optimizing Reaction Conditions

While repeating the glycosylation reaction it was observed that the yield suddenly dropped by about of 20%. A change in the colour of the commercially available promoter 4-nitrophenylsulfenyl chloride prompted to suspect a change in purity. Repeated attempts to recrystallize the compound failed. Sublimation turned out to be a much better way to purify the compound and to the best of my knowledge has not been reported before. The freshly purified compound exhibits a bright yellow colour and has a melting point between 47°C and 51°C whereas the commercially available technical grade product looks light brown and melts at 43°C. The purification did not change anything with regards to the lower yield, but allowed for ensuring a constantly good quality reagent that can now be used with 1 equivalent, instead of the 1.2 equivalents of the commercially available product. The purified compound was stored under argon in the fridge and was found to be working well even after a month of storage.

After excluding impurities and moisture in the reaction as causes for the drop in yield the only variable left was the temperature. To understand the influence of the temperature on the reaction, a special Schlenk flask with a second neck was used to measure the exact temperature of the reaction mixture at every point in time during the reaction. The activation of the donor is quick and goes to completion within 5-10 minutes even at -78°C. It was found that when adding the acceptor at temperatures lower than -60°C the aglycon transfer becomes increasingly dominant leading to the regeneration of the donor **9**. This can be observed via TLC and around 10% of the donor have been isolated after column chromatography. Under those conditions the disaccharides **13** and **28**

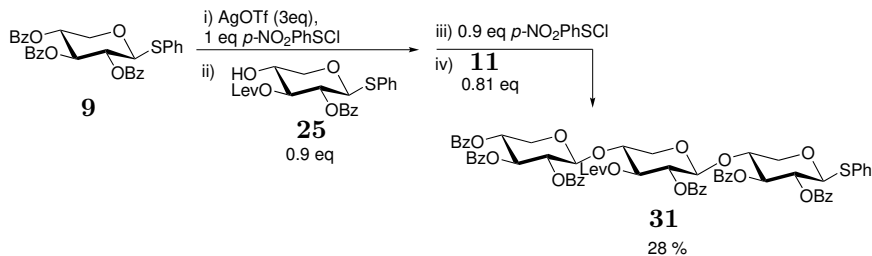
were isolated in 54% and 44% yield respectively. While it is advantageous to keep the temperature as low as possible during the activation to ensure the stability of the intermediate triflate, it is necessary to get the temperature higher than -60°C to make sure the acceptor reacts with the donor in the desired way. After several trials to examine the influence of the temperature on the reaction, the optimum reaction temperature seems to be around -55°C for the first 15 minutes (Scheme 2.19). Under these conditions yields close to 90% can be easily achieved irrespective of whether acceptor **11** or **25** is used.



Scheme 2.19: Influence of the reaction temperature on the yield.

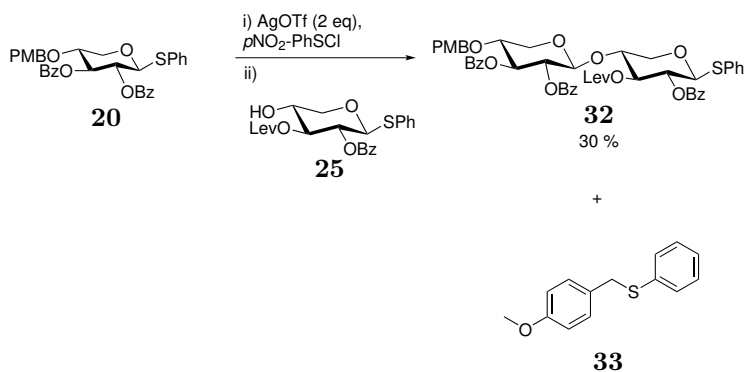
2.2.3 Synthesis of Oligoxylans

Next, the new reaction conditions were tested as a one-pot procedure with acceptors **25** and **11**. Under these conditions only 28% of the trisaccharide **31** could be isolated Scheme 2.20. Running the same reaction with the addition of TTBP²⁰⁵ to mitigate the possible influence of the increasing amount of acid lowered the yield even more to 17%, while 80% of acceptor **11** were isolated. The TLC showed a complex mixture of other products.



Scheme 2.20: One-pot procedure applied to two different acceptors.

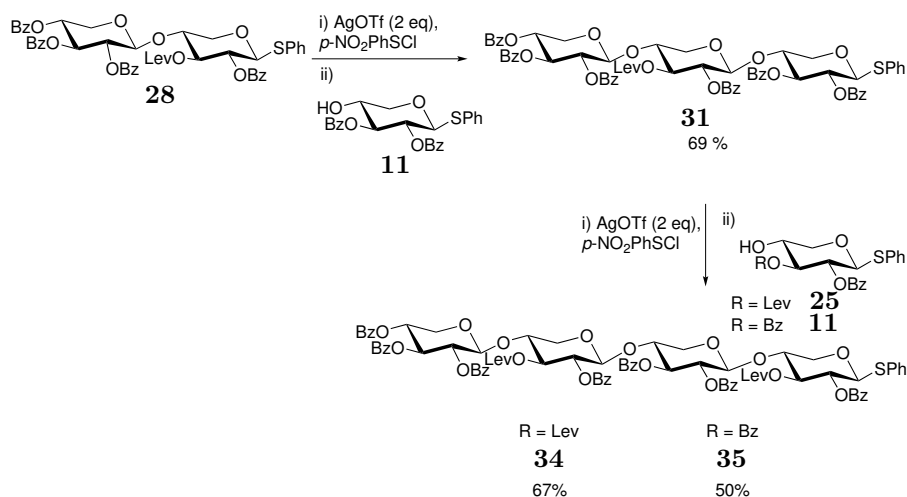
In an attempt to allow for a more convergent synthetic pathway, it was considered that using donor **20** might allow for using disaccharide **32** as a precursor to a disaccharide donor by deprotecting the PMB group after the coupling. The coupling with acceptor **25** resulted in a yield of 30%. The PMB group is not stable enough under the glycosylation conditions which then leads to a competition between acceptor **25** and the deprotected derivative of disaccharide **32**. This was confirmed when the thioether **33** was found. Its NMR spectrum matched a reference in the literature.²⁰⁶



Scheme 2.21: Disaccharide synthesis with PMB-donor

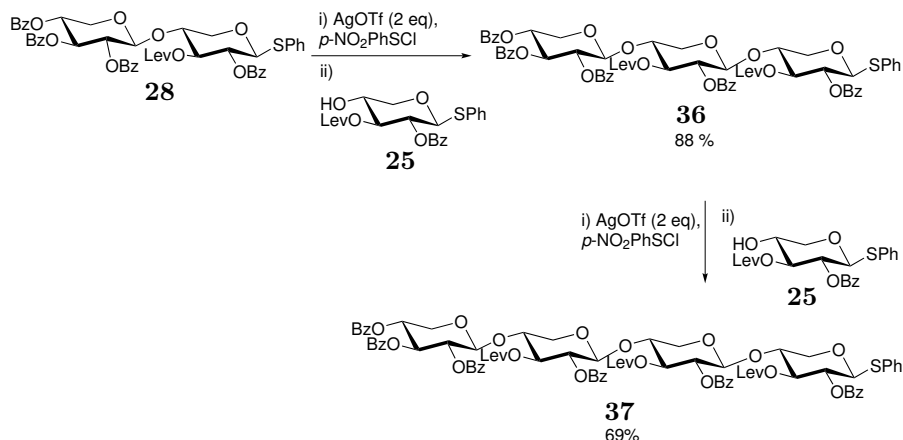
Because neither the one-pot strategy nor the convergent approach seemed viable without a major time investment in optimization, trisaccharide **31** was synthesized in a separate step after previously isolated disaccharide **28** was coupled

with acceptor **25** in 69% yield. Another round of preactivation and glycosylation of this trisaccharide with acceptor **25** yields tetrasaccharide **34** in 67% yield (Scheme 2.22). If acceptor **11** is used, tetrasaccharide **35** is obtained in 50% yield. Although less fast than the one-pot procedure, the glycosylation products once isolated can be immediately used as donors in the next glycosylation step without any further modification, thereby still saving time over approaches that do not depend on the preactivation principle.



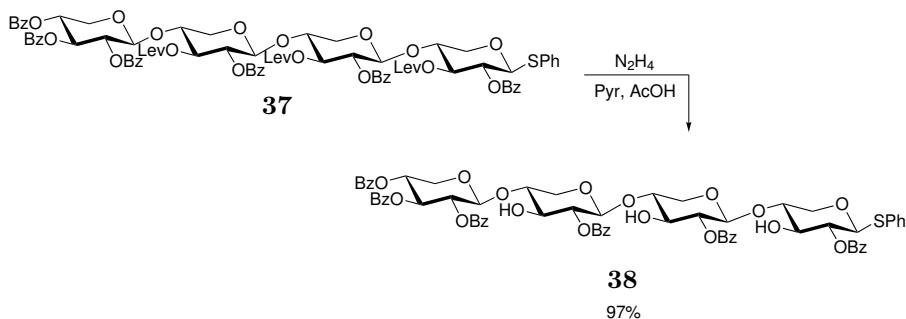
Scheme 2.22: Synthesis of tetrasaccharides **34** and **35** after isolation of the trisaccharide **31**.

In the same way, tetrasaccharide **37** can be obtained by first synthesizing trisaccharide **36** in 88% yield, followed by glycosylation with acceptor **25** to afford compound **37** in 69% yield (Scheme 2.23).



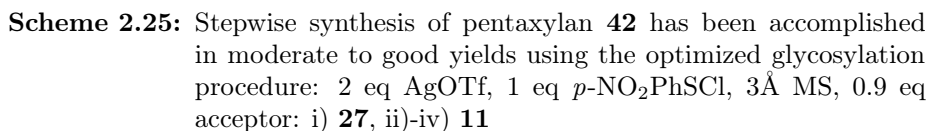
Scheme 2.23: Synthesis of tetrasaccharide **37** after isolation of the trisaccharide **36**.

The partial deprotection of the tetrasaccharide can be easily performed with hydrazine in acetic acid and pyridine leading to triol **38** in 97% yield. The purification consists of simply washing the reaction mixture.



Scheme 2.24: Removal of the levulinic esters on tetrasaccharide **37**

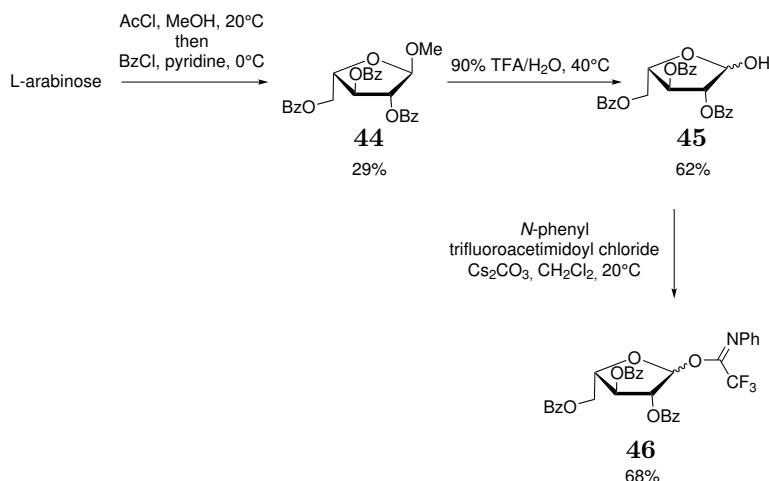
Likewise, pentasaccharide **42** has been synthesized stepwise with good to moderate yields, using acceptor **27** in the first step to synthesize the disaccharide **39**, followed by three glycosylation steps using acceptor **11** (Scheme 2.25).



Scheme 2.26: Synthesis of acceptor **43** via deprotection of the levulinic ester **42** with hydrazine.

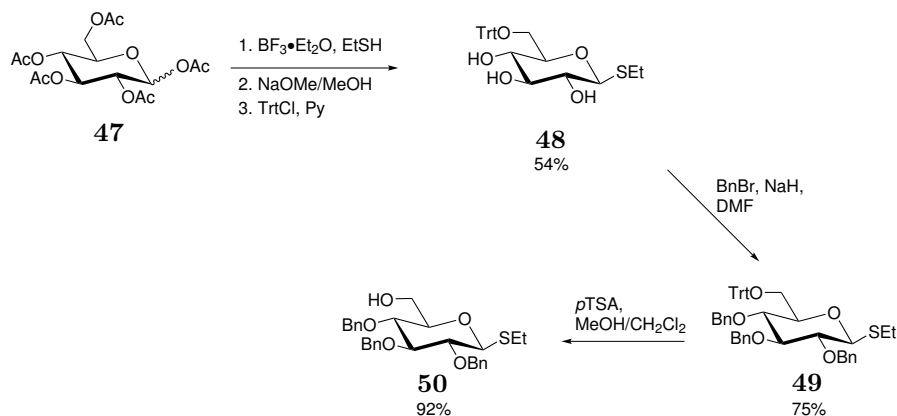
2.2.4 Synthesis of Arabinose and Glucuronic Acid Donors

After having established a reliable way to synthesize various xylan backbones, the respective arabinose and glucuronic acid donors needed to be synthesized. For arabinose the *N*-phenyl trifluoroacetimidate **46** has been chosen because of previous experience with this strategy in this department.²⁰⁷ The donor is synthesized in three steps from L-arabinose according to a procedure by Callam et al.²⁰⁸ At first, the methyl glycoside **44** is formed followed by complete benzoylation in one step. Hydrolysis leads to the hemiacetal **45**, which can then be converted to the acetimidate **46**. Compound **46** was stored in the freezer.



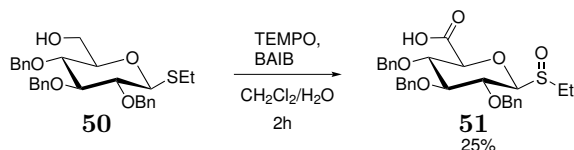
Scheme 2.27: Synthesis of arabinose *N*-phenyl trifluoroacetimidate **46**.

The glucuronic acid ester donors can be synthesized from commercially available glucose pentaacetate according to known procedures.^{209,210} The peracetylated glucose is first transformed into the thioethyl glucoside after which the acetyl groups are removed under Zemplen conditions. The primary alcohol can be selectively tritylated to give compound **48** in 54% yield over three steps. The free hydroxyl groups were then benzylated and the trityl group removed to yield the primary alcohol **50**.



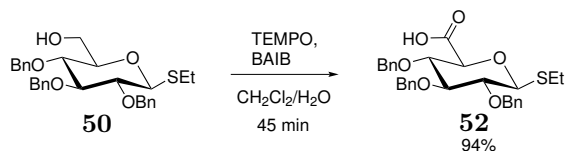
Scheme 2.28: Synthesis of glucuronic acid ester precursor **50**.

The first attempt to isolate the carboxylic acid **52** failed due to formation of the oxidation product **51** which was isolated in 25% yield as it was the most polar compound on TLC (Scheme 2.31).



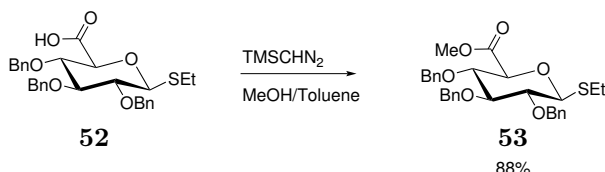
Scheme 2.29: A longer reaction time leads to oxidation of sulfur.

Reducing the reaction time to 45 minutes allowed for isolation of carboxylic acid **52** in high yield, indicating that the alcohol is more reactive towards oxidation than the thioglycoside.



Scheme 2.30: Synthesis of glucuronic acid **52**.

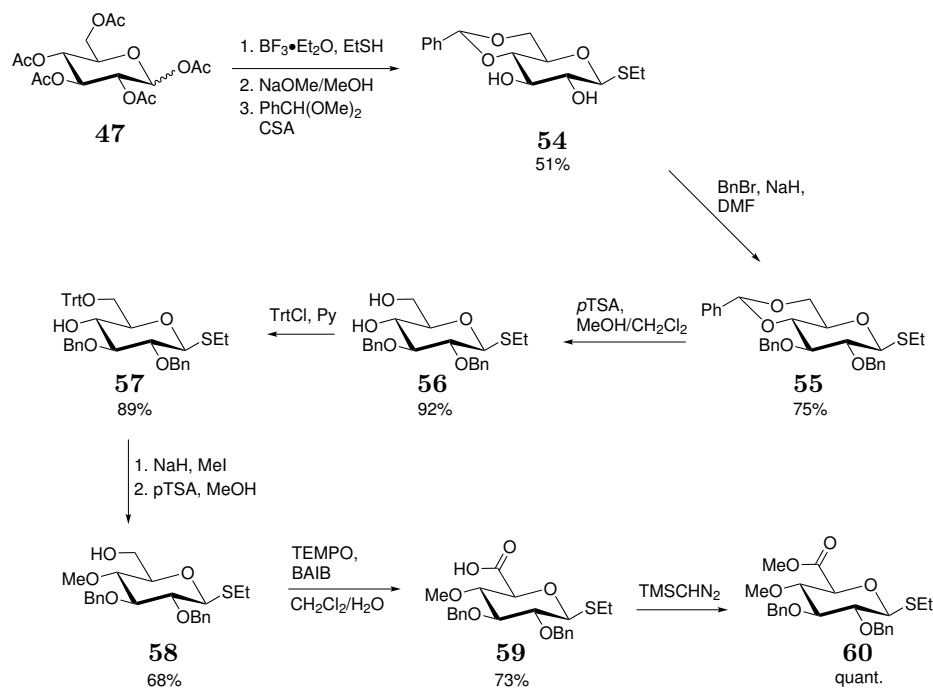
Subsequent methylation of the acid has been accomplished using trimethylsilyl-diazomethane in 88% yield. Contrary to its non-silylated cousin diazomethane, this reagent is less toxic, labile and explosive. Additionally, it has been found to give better yields in some cases.²¹¹ The great advantage of this reaction is that the only major side product is nitrogen gas and TMSOMe, leaving a clean product behind.



Scheme 2.31: Synthesis of glucuronic acid ester **53**.

In order to introduce a methyl group at the 4-position (see p. 8), acetal **54** was prepared in three steps from peracetylated glucose in 51% yield over three steps. The remaining two hydroxyl groups are benzylated and the acetal cleaved afterwards. The primary alcohol group in diol **56** can be tritylated as before, giving compound **57** with its free hydroxyl group at the 4-position.

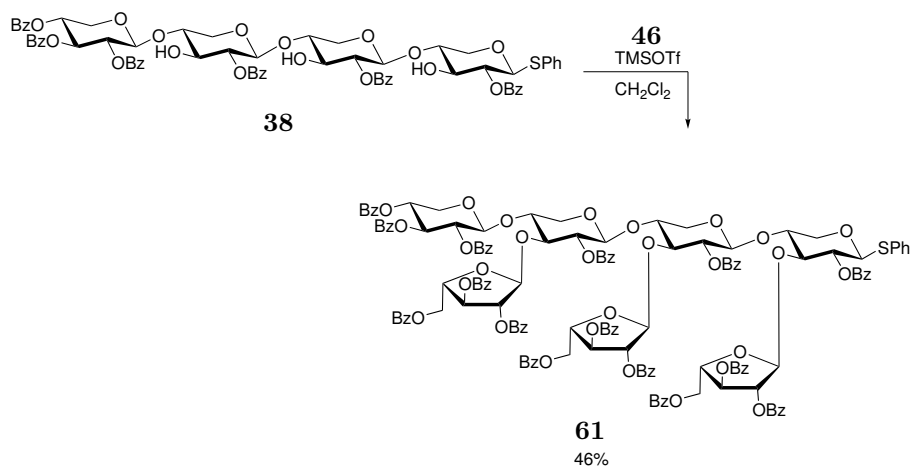
The free hydroxyl group can now be methylated using sodium hydride and methyl iodide. The trityl group is then removed again and the primary alcohol oxidized with TEMPO/BAIB.²¹² The acid **59** is methylated with trimethylsilyl-diazomethane, giving the donor **60** in quantitative yield.



Scheme 2.32: Synthesis of glucuronic acid ester **60**.

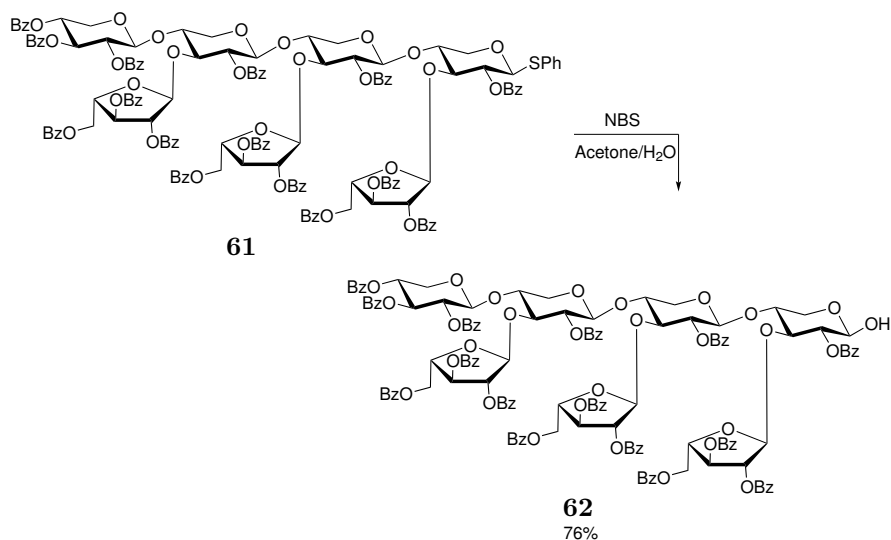
Coupling with Xylan acceptors

The attachment of arabinose to the xylan backbone has been accomplished using the arabinose donor **46**. Keeping the reaction temperature at -40°C , the reaction with xylan **38** goes to completion within less than 30 minutes when TMSOTf was used as the promoter. The heptasaccharide **61** was isolated with a yield of 46%, averaging about 77% for each of the three glycosylations.



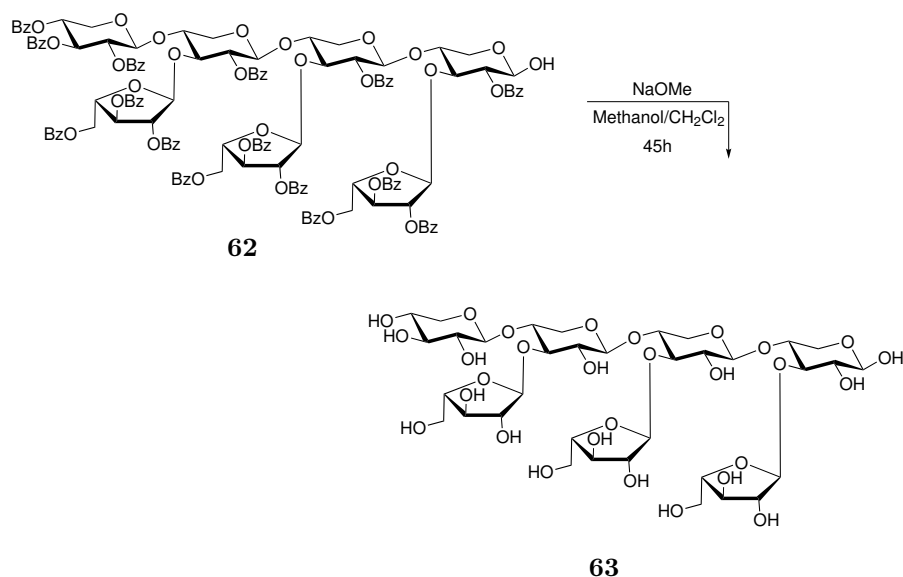
Scheme 2.33: Coupling between triol **38** and acetimidate **46** leads to formation of heptasaccharide **61**.

The anomeric thiophenyl group was removed via hydrolysis with NBS in a mixture of acetone and water, giving heptasaccharide **62** in 76% yield (Scheme 2.34).



Scheme 2.34: Removal of the thiophenyl group on the anomeric position with NBS.

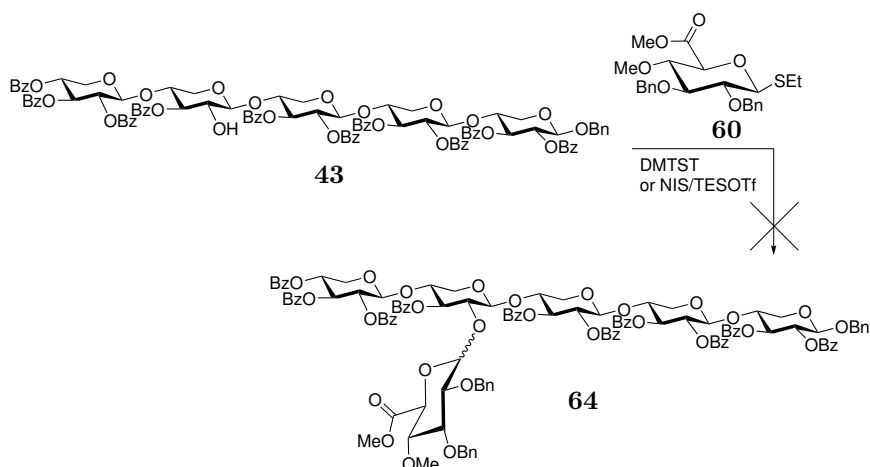
The last step consists of removing the remaining benzoyl groups. This has been done with sodium methoxide in methanol. Full consumption of UV active material was achieved after 48 hours according to TLC. HRMS acquisition confirmed that the target molecule **63** has been obtained. Unfortunately, purification via reverse phase chromatography has been problematic and the compound has not yet been fully purified. NMR analysis showed no unusual signals, such as from an elimination reaction. It is possible that the quenching of the reaction mixture led to some decomposition of the target molecule. Further work on this problem is being continued by a colleague.



Scheme 2.35: Removal of remaining benzoyl groups gives target molecule **63**

Synthesis of the glucuronoxylan was planned to be executed according to a procedure described by Oscarson and Svahnberg.¹⁹¹ In that procedure the coupling to a xylose disaccharide was accomplished using DMTST in diethyl ether with complete α -selectivity. Applying the same conditions, 1.5 equivalents of donor **60** and four equivalents of freshly prepared DMTST were mixed with acceptor **43** at 0°C. Because of the lower solubility of the acceptor, the reaction had to be conducted in a mixture of diethyl ether and dichloromethane. Contrary

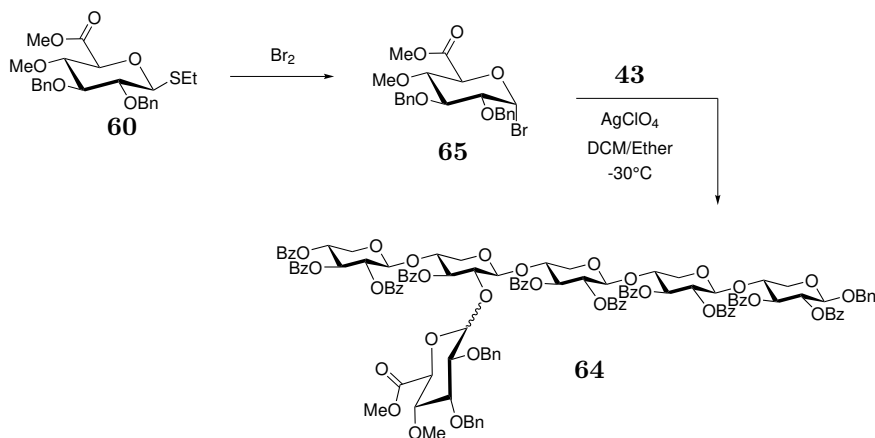
to Oscarson's 30 minutes, the reaction never went to completion. While some product formation could be observed, it was the donor that was fully consumed instead of the acceptor. Addition of more donor and DMTST did not solve that problem and even after 20 hours there was acceptor left next to several side products. Isolation of the product was attempted, but failed because of inseparable impurities. Instead of DMTST, NIS was used in the hope that the reactivity would be in favour of a coupling between acceptor and donor. However, the donor decomposed again, only this time faster than before within one hour at -10°C (Scheme 2.36).



Scheme 2.36: Coupling between donor **60** and acceptor **43** was performed under different reaction conditions. Using DMTST and NIS/TESOTf lead to many side products and decomposition of the donor. Although some product is formed, it's inseparable from the other side products.

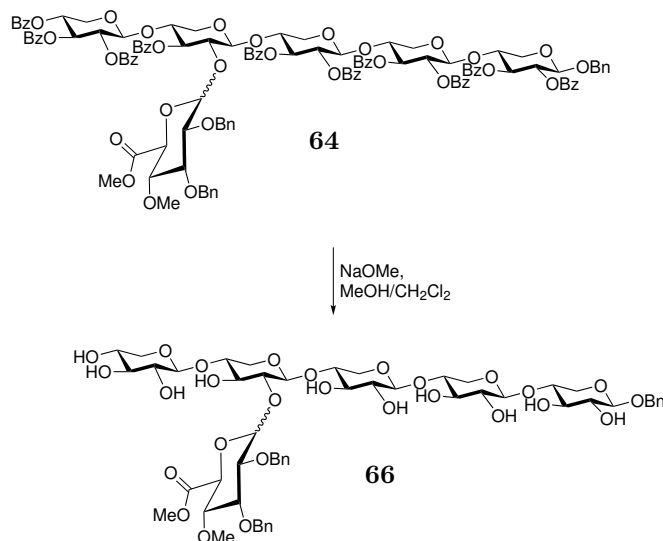
To increase the reactivity of the donor, another strategy was pursued in which the donor was reacted with bromine to give the glycosyl bromide **65**. After generating the bromide, the mixture was simply quenched and washed and the solvent removed. The crude product was then dissolved in dry dichloromethane together with the acceptor **43**. The mixture was cooled down to -30°C and silver triflate was added. This time the reaction gave a clean formation of the product. Both anomers were obtained as an inseparable mixture in a ratio of 1:1. In order

to shift the ratio towards the α -anomer, silver perchlorate was used instead of silver triflate and 10% ether was added as solvent (Any more ether leads to precipitation of the acceptor.). This resulted in isolation of both anomers in a ratio of 7:3 and a yield of 64%. Also, 15% of the acceptor was recovered, giving an adjusted yield of 75%.



Scheme 2.37: Thioglycoside **60** was brominated and then coupled with AgClO_4 to give a mixture of anomers (7 α : 3 β) in 75% yield brsm.

Since the anomeric mixture is inseparable, the hexasaccharide **64** was debenzoylated so that the two anomers might be separated via reverse-phase chromatography.



Scheme 2.38: Hexasaccharide **64** was deprotected with sodium methoxide to give polyol **66**. Purification of this compound has so far been unsuccessful.

A first attempt to separate the mixture by HPLC did not succeed and gave only an impure mixture of compounds. Additionally there have been technical problems with the ELS detector. The presence of the compound could be verified via HRMS. Further work on this problem is being continued by a colleague.

2.3 Conclusion

It has been shown that the step-wise glycosylation method based on preactivation is a viable path towards arabinoxylans as well as glucuronoxylans. The building blocks are easily accessible and the method allows for a rapid assembly of at least pentaxylans. While investigating the synthetic strategy to build the xylan backbone the following key results have been obtained:

- A better understanding has been gained on how the reaction temperature

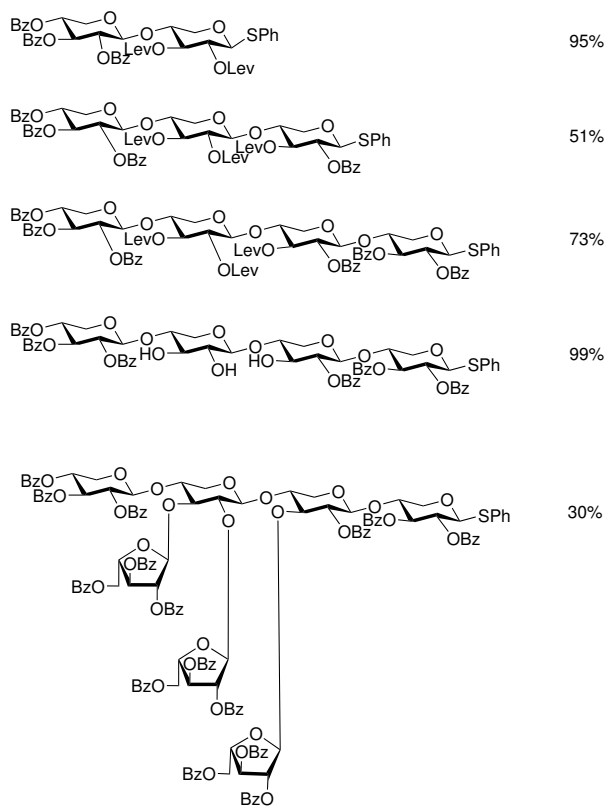
influences the competition between aglycon transfer and glycosylation.

- A new way to purify the promoter by sublimation has been found.
- A new protocol has been established that allows the synthesis of oligoxylans with up to at least five units in consistently high yields.

Furthermore it has been shown that the chosen protecting group strategy allows for an easy deprotection of the xylan backbone. It also gives one both options on where to install the branching sugars. It has been shown that either the O-2 or O-3 position are easily accessible and conceivably both positions could be accessed at the same time. Attaching arabinose or glucuronic acid donors to the backbone has been accomplished via the acetimidate method and the Koenigs-Knorr coupling, respectively. While the arabinoxylan has been completely deprotected, there remains the challenge of purifying the reaction mixture. In the same way, the purification of the single α -anomer of the glucuronoxylan needs to be optimized.

2.4 Future Work

This project is being continued by two coworkers in our group. As of now, the strategy has been extended towards utilizing xylose building blocks containing two Lev-groups on the O-2 as well as O-3 position. The following oligosaccharides have so far been synthesized (yields refering to last synthetic step):²¹³



The work is being continued towards the synthesis of a series of new arabinoxylans with different substitution patterns. Furthermore, the purification of the (partially) deprotected oligosaccharides is currently being investigated.

Experimental Data

3.1 General

All material, reagents and solvents were purchased from Alfa Aesar, Carbosynth, Sigma-Aldrich or TCI chemicals and used without further purification unless specified otherwise. All solvents were HPLC-grade. The dry solvents were obtained from an Innovative Technology PS-MD-7 Pure-solv solvent purification system. Reactions requiring anhydrous conditions were carried out in flame-dried glassware under inert atmosphere, either using argon or nitrogen. Solvents were removed under vacuum at 30°C. All reactions were monitored by thin-layer chromatography (TLC), performed on Merck aluminum plates precoated with 0.25 mm silica gel 60 F254. Compounds were visualized under UV irradiation and/or heating after applying a solution of $\text{Ce}(\text{SO}_4)_2$ (2.5 g) and $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$ (6.25 g) in 10% aqueous H_2SO_4 (250 ml). Column chromatography was performed using Geduran silica gel 60 with specified solvents given as volume ratio. 1D (^1H and ^{13}C) and 2D (gCOSY, HSQC, HMBC) NMR spectra were recorded on a Bruker Ascend 400 or a Varian Mercury 300 spectrometer. 2D NMR experiments

were performed in order to elucidate the carbohydrate structures. Optical rotations were measured with a Perkin-Elmer Model 241 Polarimeter with a path length of 1 dm. High-resolution mass spectrometry (HRMS) data were recorded on a Bruker Solarix XR 7T ESI/MALDI-FT-ICR MS, with external calibration performed using NaTFA cluster ions. The elemental analyses were performed at the Microanalytic Laboratory Kolbe in Mülheim an der Ruhr (Germany).

3.2 Experimental Procedures and Analytical Data

General Procedure I - Tin-Mediated Glycosylation with Perbenzoylated Glycosyl Bromide

A suspension of the unprotected acceptor **1** (0.5 mmol) and Bu_2SnO (0.75 mmol) in MeOH (3.0 ml) was refluxed until a clear solution was obtained (3 h). The solvent was evaporated in vacuo followed by drying at high vacuum for 2 h to give the stannylene derivative as a colorless foam. The bromide donor (0.9 mmol) and 4 Å MS (500 mg) were added to a solution of the stannylene derivative in CH_2Cl_2 (5 ml). The suspension was stirred at $-30\text{ }^\circ\text{C}$ for 30 min. AgOTf (0.9 mmol) was then added, and the mixture was stirred in the dark while the temperature was allowed to reach $10\text{ }^\circ\text{C}$. After 6 h the mixture was filtered, diluted with CH_2Cl_2 , washed once with 2 M aqueous HCl, once with saturated aqueous NaHCO_3 and once with water. The organic layer was dried (MgSO_4), filtered and concentrated. The crude product was purified by column chromatography (toluene/ acetone, 9:1).

General Procedure II - Glycosylation via Preactivation of Thiophenylglycoside

To a two-necked 100 ml Schlenk flask are added 1 g powdered 3 Å molecular sieves and a stirring bar. The necks are fitted with a stopper and a septum. After flame drying the flask under vacuum and filling it with argon, a solution of

0.361 mmol of donor in 3 ml dichloromethane and 2 equivalents of silver triflate in 3 ml of toluene are added. The solution is left to stir in the dark for 15 minutes and then cooled down to -60°C . One equivalent of *p*-nitrophenylsulfenyl chloride in 1 ml of dichloromethane is added quickly and the solution is left to stir for 5 minutes. Afterwards a solution of 0.9 equivalents of the acceptor are added quickly and the temperature of the solution is kept between -45°C and -55°C for 15 minutes after which it is left to warm up to -15°C . The mixture is subsequently quenched with 3 equivalents of triethylamine, filtered through a pad of Celite and purified via flash chromatography.

General Procedure III - Glycosylation with *N*-Phenyl Trifluoroacetimidate donor

A mixture of the acceptor (0.08 mmol) and donor (1.1 equivalents per alcohol group in acceptor) was co-evaporated with toluene and subjected to high vacuum for 2h. The mixture was dissolved in 20 ml anhydrous CH_2Cl_2 and cooled to -40°C . Trimethylsilyl trifluoromethanesulfonate (0.1 eq.) was added and the reaction mixture was stirred at -40°C until TLC (toluene/EtOAc 20:1) showed completion of the reaction (10-30 min). The reaction mixture was quenched by addition of 0.7 eq. triethylamine, evaporated and purified by flash column chromatography.

General Procedure IV - Deprotection of PMB-group

The PMB-ether (5.66 mmol) was dissolved in $\text{CH}_2\text{Cl}_2/\text{H}_2\text{O}$ (10:1, 30 ml), followed by addition of DDQ (1.927 g, 8.49 mmol). The resulting mixture was stirred at room temperature overnight. After the reaction was completed, it was diluted with CH_2Cl_2 , filtered through a pad of Celite, washed with a saturated aqueous solution of NaHCO_3 until the aqueous phase assumed a light yellow color, dried over magnesium sulfate and purified by column chromatography.

General Procedure V - Deprotection of Lev-group

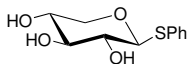
The levulinic ester was dissolved in pyridine to provide a 1.4M solution. N+1 equivalents¹ of 50% hydrazine hydrate in a 1M solution of pyridine/acetic acid (2:1) are added to the solution. The reaction mixture is left to stir at room temperature for 10 minutes, after which TLC indicated full consumption of the starting material. Acetone (500 eq.) is added and the mixture is left to stir for another 20 minutes. After addition of ethyl acetate, the solution is washed with 10% HCl, saturated NaHCO₃ and water. The organic phase is dried over magnesium sulfate and the solvent removed in vacuo.

Purification of 4-nitrophenylsulfenyl chloride

A 100 ml Schlenk flask is filled with 2 g of 95% 4-nitrophenylsulfenyl chloride (technical grade, melting point 43°C). The compound is sublimated at 35°C and 0.07 mbar. After three days 920 mg (46%) of the bright yellow product have been obtained with a melting point of 47-51°C.

Synthesis of DMTST

DMTST was synthesized using methyl triflate and dimethyl disulfide according to a literature procedure.²¹⁴

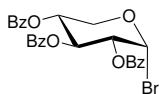


(1) Phenyl 1-thio-β-D-xylopyranoside

D-xylose (50.0 g, 0.333 mol) was suspended in dichloromethane (250 ml) together with Et₃N (231 ml, 1.67 mol) and DMAP (8.1 g, 0.067 mol), then acetic anhydride (126 ml, 1.33 mol) was added at 0°C. The reaction was stirred until TLC indicated full conversion within 6 hours. The reaction mixture was washed with ice-water, 300 ml of 1 M HCl and brine (200 ml). The organic layers were dried over Na₂SO₄, filtered and evaporated under reduced pressure. The crude, without further purification, was dissolved in dichloromethane (300 ml). The stirred mixture

¹N = number of Lev-groups in the molecule

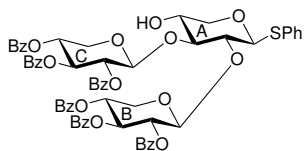
was cooled to 0 °C and thiophenol (41 ml, 0.400 mol) and $\text{BF}_3 \cdot \text{OEt}_2$ (122 ml, 0.999 mol) were added, under inert atmosphere. The solution was stirred at room temperature until disappearance of the starting material on TLC, then diluted with dichloromethane and washed successively with saturated sodium hydrogen carbonate (2x250 ml) and water (2x150 ml), dried over Na_2SO_4 , filtrated and concentrated in vacuo. The residue was dissolved in methanol (200 ml) and a 0.1 M solution of sodium methoxide in methanol was added. After 15 min the mixture was neutralized with Amberlite IR-120(H+) resin, filtered, and concentrated under reduced pressure. The crude was recrystallized in ethyl acetate / heptane to yield **1** (29.5 g, 37%) as a white solid. $^1\text{H-NMR}$ (400 MHz, MeOD) δ 7.57 – 7.48 (m, 2H, ArH), 7.37 – 7.23 (m, 3H, ArH), 4.57 (d, $J = 9.3$ Hz, 1H, H-1), 3.95 (dd, $J = 11.3, 5.2$ Hz, 1H, H-5), 3.49 (ddd, $J = 10.0, 8.8, 5.2$ Hz, 1H, H-4), 3.36 (t, $J = 8.6$ Hz, 1H, H-3), 3.24 (dd, $J = 11.3, 10.1$ Hz, 1H, H-5), 3.22 (dd, $J = 9.3, 8.5$ Hz, 1H, H-2). $^{13}\text{C-NMR}$ (101 MHz, MeOD) δ 133.5, 131.7, 128.5, 127.1, 88.7 (C-1), 77.8 (C-3), 72.3 (C-2), 69.5 (C-4), 69.0 (C-5). The data are in accordance with literature.²¹⁵



(2) 2,3,4-Tri-*O*-benzoyl- α -D-xylopyranosyl

bromide The compound was prepared according to a known procedure and the analytical data matched the literature values.^{216,217} $^1\text{H-NMR}$ (300 MHz, CDCl_3)

δ 8.04 – 7.91 (m, 5H), 7.59 – 7.30 (m, 10H), 6.82 (d, $J = 3.9$ Hz, 1H, H-1), 6.24 (t, $J = 9.8$ Hz, 1H), 5.55 – 5.44 (m, 1H), 5.29 (dd, $J = 9.9, 4.0$ Hz, 1H), 4.36 (dd, $J = 11.3, 5.9$ Hz, 1H), 4.14 (t, $J = 11.1$ Hz, 1H). $^{13}\text{C-NMR}$ (75 MHz, CDCl_3) δ 165.7, 165.6, 165.5, 133.9, 133.8, 133.5, 130.2, 130.0, 129.9, 129.1, 128.8, 128.7, 128.7, 128.5, 88.0, 71.6, 70.1, 69.0, 63.1.



(3) Phenyl 2,3,4-tri-*O*-benzoyl- β -D-xylopyran-

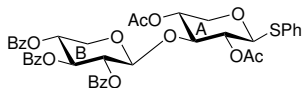
osyl-(1 \rightarrow 3)-[2,3,4-tri-*O*-benzoyl- β -D-xylopyra-

nosyl-(1 \rightarrow 2)]-1-thio- β -D-xylopyranoside This

compound was synthesized according to General Procedure I. $^1\text{H-NMR}$ (300 MHz, CDCl_3) δ 8.03 –

7.94 (m, 12H), 7.58 – 7.44 (m, 7H), 7.43 – 7.31 (m,

13H), 7.15 – 7.06 (m, 2H), 6.96 (m, 1H), 5.87 (t, $J = 8.4$ Hz, 1H, C-3), 5.80 (t, $J = 7.6$ Hz, 1H, B-3), 5.50 – 5.33 (m, 4H, B-2, B-4, C-2, C-4), 5.20 (d, $J = 5.5$ Hz, 1H, B-1), 4.90 (d, $J = 6.4$ Hz, 1H, C-1), 4.83 (d, $J = 4.5$ Hz, 1H, A-1), 4.58 – 4.51 (m, 2H, C-5a, B-5a), 4.28 (dd, $J = 12.5, 2.6$ Hz, 1H, A-5a), 4.06 (t, $J = 5.2$ Hz, 1H, A-3), 3.96 – 3.94 (m, 1H, A-4), 3.79 – 3.60 (m, 4H, C-5b, B-5b, A-2), 3.41 (dd, $J = 12.3, 5.3$ Hz, 1H, A-5b). $^{13}\text{C-NMR}$ (75 MHz, CDCl_3) δ 165.7, 165.7, 165.7, 165.6, 165.6, 165.4, 133.6, 133.5, 133.4, 133.3, 130.7, 130.1, 130.0, 130.0, 129.9, 129.6, 129.3, 129.3, 129.3, 129.2, 129.0, 128.8, 128.6, 128.6, 128.5, 128.4, 127.0, 99.9 (C-1), 99.2 (B-1), 88.1 (A-1), 76.1 (A-3), 73.9 (A-4), 71.3 (C-4), 71.1 (C-3), 70.8 (B-3), 70.8 (C-2), 70.7 (A-2), 69.5 (B-4), 69.3 (B-2), 62.6 (B-5), 61.9 (C-5), 61.1 (A-5).



(4) Phenyl 2,3,4-tri-*O*-benzoyl- β -D-xy-

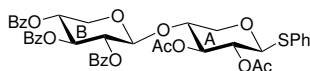
lopyranosyl-(1 \rightarrow 3)-2,4-di-*O*-acetyl-1-

thio- β -D-xylopyranoside This compound

was prepared according to General Procedure

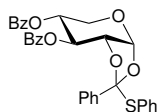
I, using acetonitrile as solvent during the glycosylation. To allow for full characterization, the remaining hydroxyl groups were acetylated by mixing the compound with acetyl chloride in pyridine over 24 hours. $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.08 – 8.01 (m, 2H), 8.00 – 7.96 (m, 4H), 7.59 – 7.49 (m, 3H), 7.44 – 7.34 (m, 4H), 7.36 – 7.27 (m, 2H), 7.25 – 7.20 (m, 5H), 5.72 (t, $J = 6.1$ Hz, 1H, B-3), 5.37 (dd, $J = 6.1, 4.4$ Hz, 1H, B-2), 5.28 (td, $J = 5.5, 3.8$ Hz, 1H, B-4), 5.13 (d, $J = 4.3$ Hz, 1H, B-1), 5.04 – 4.98 (m, 2H, A-1, A-2), 4.96 (td, $J = 5.9, 3.9$ Hz, 1H, A-4a), 4.48 (dd, $J = 12.5, 3.7$ Hz, 1H, B-5a), 4.40 (dd, $J = 12.4, 3.7$ Hz, 1H, A-5a), 4.07 (t, $J = 5.7$ Hz, 1H, A-3), 3.80 (dd, $J = 12.4, 5.4$ Hz, 1H, B-5b), 3.52 (dd, $J = 12.4, 5.8$ Hz, 1H, A-5b), 2.11 (s, 3H, Ac), 2.06 (s, 3H, Ac). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 170.0 (Ac), 169.6 (Ac), 165.7 (Bz), 165.3 (Bz), 165.2 (Bz), 134.5, 133.6, 133.4, 131.3, 130.1, 130.1, 130.0, 129.5, 129.4, 129.2,

128.9, 128.6, 128.5, 127.5, 99.3 (B-1), 86.1 (A-1), 74.8 (A-3), 70.3 (A-2), 69.6 (B-2), 69.4 (B-3), 69.1 (A-4), 68.7 (B-4), 62.6 (A-5), 60.9 (B-5), 21.2 (Ac), 21.1 (Ac).



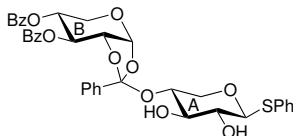
(5) Phenyl 2,3,4-tri-*O*-benzoyl- β -D-xylopyranosyl-(1 \rightarrow 4)-2,3-di-*O*-acetyl-1-thio- β -D-xylopyranoside This compound

was prepared according to General Procedure I, using acetonitrile as solvent during the glycosylation. To allow for full characterization, the remaining hydroxyl groups were acetylated by mixing the compound with acetyl chloride in pyridine over 24 hours. **$^1\text{H-NMR}$** (300 MHz, CDCl_3) δ 8.03 – 7.99 (m, 2H), 7.97 – 7.93 (m, 4H), 7.59 – 7.49 (m, 3H), 7.48 – 7.32 (m, 7H), 7.33 – 7.27 (m, 3H), 7.17 (m, 1H), 5.72 (t, J = 6.9 Hz, 1H, B-3), 5.29 – 5.17 (m, 3H, A-3, B-2, B-4), 4.92 – 4.85 (m, 2H, A-2, B-1), 4.68 (d, J = 9.2 Hz, 1H, A-1), 4.40 (dd, J = 12.2, 4.0 Hz, 1H, B-5a), 4.09 (dd, J = 11.8, 5.1 Hz, 1H, A-5a), 3.89 (td, J = 9.4, 5.2 Hz, 1H, A-4), 3.70 (dd, J = 12.2, 6.5 Hz, 1H, B-5b), 3.32 (dd, J = 11.7, 9.8 Hz, 1H, B-5a), 2.08 (s, 2H, Ac), 2.06 (s, 3H, Ac). **$^{13}\text{C-NMR}$** (75 MHz, CDCl_3) δ 170.1 (Ac), 169.7 (Ac), 165.7 (Bz), 165.4 (Bz), 165.1 (Bz), 133.6, 132.9, 130.0, 130.0, 129.9, 129.1, 128.6, 128.4, 125.4, 99.8 (B-1), 86.5 (A-1), 75.3 (A-4), 73.7 (A-3), 70.4 (A-2, B-2), 69.9 (B-3), 68.9 (B-4), 66.7 (A-5), 61.3 (B-5), 21.04 (Ac), 20.96 (Ac).



(6) 3,4-Di-*O*-benzoyl-1,2-*O*-(phenylthiobenzylidene)- α -D-xylopyranose **$^1\text{H-NMR}$** (300 MHz, CDCl_3) δ 8.05 – 7.97 (m, 5H), 7.91 – 7.88 (m, 2H),

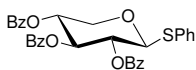
7.59 – 7.49 (m, 4H), 7.41 – 7.29 (m, 9H), 5.69 (t, J = 5.4 Hz, 1H, H-3), 5.42 – 5.37 (m, 2H, H-1, H-2), 5.19 – 5.15 (m, 1H, H-4), 4.41 (dd, J = 12.6, 3.2 Hz, 1H, H-5a), 3.81 (dd, J = 12.6, 4.7 Hz, 1H, H-5b). **$^{13}\text{C-NMR}$** (75 MHz, CDCl_3) δ 165.7, 165.4, 133.7, 133.6, 130.2, 130.1, 130.1, 129.3, 129.0, 128.7, 110.0 ($\text{PhC(O)}_2\text{SPh}$), 94.9 (C-1), 69.2 (C-3), 68.6 (C-2), 68.2 (C-4), 54.7 (C-5).



(8) 3,4-di-*O*-benzoyl- α -D-xylopyranose-1,2-di-yl(1-*O*-phenylthio- β -D-xylopyranoside)-4-yl

orthobenzoate $^1\text{H-NMR}$ (300 MHz, CDCl_3) δ 7.98 (d, $J = 7.2$ Hz, 2H), 7.80 (d, $J = 7.3$ Hz, 2H), 7.66 – 7.61 (m, 2H), 7.54 – 7.45 (m, 2H), 7.41 – 7.36 (m,

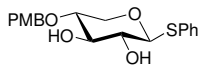
4H), 7.35 – 7.24 (m, 3H), 7.21 – 7.17 (m, 3H), 7.11 – 7.06 (m, 2H), 5.92 (d, $J = 4.7$ Hz, 1H, B-1), 5.62 (t, $J = 2.7$ Hz, 1H, B-3), 5.21 – 5.15 (m, 1H, B-4), 4.66 – 4.63 (m, 1H, B-2), 4.39 (d, $J = 9.2$ Hz, 1H, A-1), 4.04 (dd, $J = 12.2$, 6.5 Hz, 1H B-5a), 3.87 (dd, $J = 11.5$, 4.5 Hz, 1H, A-5a), 3.56 – 3.44 (m, 3H, A-3, A-4, B-5b), 3.24 (td, $J = 9.1$, 2.6 Hz, 1H, A-2), 3.13 (dd, $J = 11.5$, 9.5 Hz, 1H, A-5b), 3.03 (d, $J = 1.7$ Hz, 1H, OH), 2.79 (d, $J = 2.6$ Hz, 1H, OH). $^{13}\text{C-NMR}$ (75 MHz, CDCl_3) δ 165.4 (Bz), 164.9 (Bz), 135.5, 133.9, 133.5, 132.6, 132.3, 130.1, 130.0, 129.9, 129.1, 128.9, 128.7, 128.5, 128.3, 128.2, 126.3, 125.4, 121.6 ($\text{PhC}(\text{O})_3$), 97.3 (B-1), 88.8 (A-1), 76.0 (A-4), 73.7 (B-2), 72.3 (A-2), 71.9 (A-3), 68.9 (B-3), 67.9 (A-5), 67.8 (B-4), 59.9 (B-5).



(9) Phenyl 2,3,4-tri-*O*-benzoyl-1-thio- β -D-xylopyranoside

The triol **1** (5.12 g, 21.13 mmol) was dissolved in pyridine (45 ml) and BzCl (7.4 ml, 63.40 mmol). The reaction mixture was stirred at room

temperature for 1 h and the excess of BzCl was quenched by adding 10 ml of methanol and the mixture was stirred for additional 10 minutes. The disappearance of a white precipitate was observed. The reaction mixture was diluted with dichloromethane and washed with 1 M HCl (2x100 ml) and water (2x100 ml). The organic phase was dried over Na_2SO_4 , filtered and the solvent evaporated in vacuo. The residue was purified by column chromatography to afford **9** (5.1 g, 84%). $R_f = 0.28$ (3 ethyl acetate / 7 heptane) $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.06 – 8.03 (m, 2H), 8.01 – 7.98 (m, 4H), 7.56 – 7.51 (m, 5H), 7.42 – 7.32 (m, 9H), 5.79 – 5.76 (m, 1H), 5.48 – 5.45 (m, 1H), 5.32 – 5.27 (m, 2H), 4.71 (dd, $J = 12.3$, 4.0 Hz, 1H), 3.83 (dd, $J = 12.3$, 6.5 Hz, 1H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 165.6, 165.3, 165.3, 133.6, 133.5, 133.5, 133.2, 132.8, 130.2, 130.1, 130.1, 129.3, 129.2, 129.0, 128.6, 128.6, 128.5, 128.3, 86.5, 70.6, 70.1, 68.8, 63.7. **Elemental Analysis:** calc. C: 69.30 H: 4.73 S: 5.78; found: C: 69.26 H: 4.70 S: 5.66

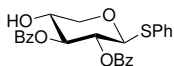


(10) Phenyl 4-*O*-*p*-methoxybenzyl-1-thio-β-D-xylopyranoside Phenyl thioxyloside **1** (29.5 g, 0.122

mol) was solubilized in DMF (200 ml) with CSA (2.83 g, 0.012 mol) and 2-methoxypropene (37.3 ml, 0.366 mol). The reaction was stirred at 60 °C for 1 hour then cooled to room temperature and quenched with Et₃N (30 ml). The solvent was evaporated and the residue purified by column chromatography (heptane/ethyl acetate/CH₂Cl₂ 4:1:1). 24.1 g (70%) of the phenyl 2,3-*O*-isopropylidene 1-thio-β-D-xylopyranoside was isolated as a colorless oil.²⁰⁰ ¹H-NMR (400 MHz, CDCl₃) δ 7.56 – 7.48 (m, 2H, ArH), 7.36 – 7.27 (m, 3H, ArH), 4.51 (d, *J* = 9.4 Hz, 1H, H-1), 4.11 (dd, *J* = 11.2, 5.2 Hz, 1H, H-5), 3.71 (ddd, *J* = 10.3, 8.8, 5.2 Hz, 1H, H-4), 3.55 (t, *J* = 8.7 Hz, 1H, H-3), 3.34 (dd, *J* = 9.4, 8.6 Hz, 1H, H-2), 3.32 (dd, *J* = 11.2, 10.3 Hz, 1H, H-5), 2.17 (s, 6H, 2xCH₃). ¹³C-NMR (101 MHz, CDCl₃) δ 133.0, 132.0, 129.0, 128.3, 111.5, 85.6 (C-1), 83.0i (C-3), 75.3 (C-2), 70.0 (C-4), 69.1 (C-5), 26.8 (CH₃), 26.7 (CH₃). The data are in accordance with the literature.²¹⁹

A solution of the previously isolated compound (17.5 g, 62 mmol), PMBCl (10.9 ml, 80.6 mmol) and NaH (60% oil dispersion, 3.0 g, 74.4 mmol) in DMF (120 ml) was stirred for 16 h at room temperature, then quenched with 10% HCl solution (28 ml). The reaction mixture was diluted with dichloromethane (100 ml) and washed with NaHCO₃ (300 ml) and successively brine (200 ml). The organic layers were collected and dried over Na₂SO₄, filtered and concentrated in vacuo. The crude compound was dissolved in CH₂Cl₂/CH₃OH (1:1, 200 ml) and stirred with CSA (14.4 g, 62 mmol) at room temperature overnight. When complete conversion was observed, the reaction was quenched by Et₃N and concentrated. Silica gel purification (6 heptane : 4 ethyl acetate, *R_f* 0.17) afforded **10** (20.5 g, 91%). $[\alpha]_D^{20} = -59$ (c 1.0, CHCl₃), ¹H-NMR (400 MHz, CDCl₃) δ 7.59 – 7.46 (m, 2H, ArH), 7.35 – 7.21 (m, 5H, ArH), 6.93 – 6.83 (m, 2H, ArH), 4.61 (d, *J* = 11.2 Hz, 1H, OCH₂Ph), 4.57 (d, *J* = 11.2 Hz, 1H, OCH₂Ph), 4.55 (d, *J* = 8.9 Hz, 1H, H-1), 4.06 (dd, *J* = 11.5, 4.8 Hz, 1H, H-5), 3.80 (s, 3H, OCH₃), 3.66 (t, *J* = 8.6 Hz, 1H, H-3), 3.46 (ddd, *J* = 9.6, 8.6, 4.8 Hz, 1H, H-4), 3.40 (t, *J* = 8.6 Hz, 1H, H-2), 3.27 (dd, *J* = 11.5, 9.7 Hz, 1H, H-5). ¹³C-NMR (101 MHz, CDCl₃) δ 159.7, 132.8, 132.2, 130.0, 129.7, 129.2, 128.3, 127.6, 114.1, 88.8 (C-1), 76.6 (C-4), 76.5 (C-3), 72.8 (OCH₂Ph), 72.1 (C-2), 67.1 (C-5), 55.4 (OCH₃). HRMS (MALDI) *m/z* calcd for C₁₉H₂₂O₅S

(M+Na⁺) 385.1080, found 385.1090.



(11) Phenyl 2,3-di-*O*-benzoyl-1-thio- β -D-xylo-

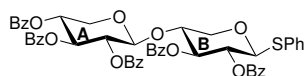
pyranoside The diol **10** (0.680 g, 1.88 mmol) was dissolved in pyridine (5 ml) and BzCl (0.436 ml, 3.75 mmol) was added. The reaction mixture was stirred

at 22 °C for 2 h, then it was diluted with dichloromethane and washed with 1 M HCl (2x20 ml) and water (2x20 ml). The organic phase was dried over Na₂SO₄, filtered and the solvent removed under vacuum. The residue was purified by column chromatography to afford the dibenzoylated product which is used in the next step. (0.877 g, 82%). $[\alpha]_D^{20} = + 55.0$ (c 1.0, CHCl₃). **¹H-NMR** (400 MHz, CDCl₃) δ 7.99 – 7.95 (m, 4H), 7.56 – 7.51 (m, 2H), 7.50 – 7.46 (m, 2H), 7.42 – 7.34 (m, 4H), 7.31 – 7.27 (m, 3H), 7.15 – 7.12 (m, 2H), 6.74 – 6.71 (m, 2H), 5.60 (t, $J = 8.0$ Hz, 1H, H-3), 5.34 (t, $J = 8.0$ Hz, 1H, H-2), 5.04 (d, $J = 8.1$ Hz, 1H, H-1), 4.55 (d, $J = 11.8$ Hz, 1H, OCH₂Ph), 4.52 (d, $J = 11.8$ Hz, 1H, OCH₂Ph), 4.28 (dd, $J = 11.9$, 4.6 Hz, 1H, H-5), 3.79 – 3.74 (m, 1H, H-4), 3.75 (s, 3H, OCH₃), 3.57 (dd, $J = 11.9$, 8.5 Hz, 1H, H-5). **¹³C-NMR** (101 MHz, CDCl₃) δ 165.7 (Bz), 165.4 (Bz), 159.5, 133.4, 133.2, 132.5, 130.1, 130.0, 129.7, 129.7, 129.5, 129.4, 129.1, 128.5, 128.5, 128.1, 113.9, 86.9 (C-1), 73.9 (C-3), 73.6 (C-4), 72.5 (OCH₂Ph), 70.6 (C-2), 66.4 (H-5), 55.3 (OCH₃). **HRMS (MALDI)** m/z calcd for C₃₃H₃₀O₇S (M+Na⁺) 593.1604, found 593.1617.

The compound **20** obtained in the previous step was converted to the acceptor **11** according to General Procedure IV and was obtained as a white solid in 87% yield after column chromatography (heptane/ethyl acetate 7:3, **R_f** 0.18). $[\alpha]_D^{20} = + 63.7$ (c 1.0, CHCl₃). **¹H-NMR** (400 MHz, CDCl₃) δ 8.07 – 7.97 (m, 4H, ArH), 7.58 – 7.47 (m, 4H, ArH), 7.45 – 7.38 (m, 4H, ArH), 7.34 – 7.29 (m, 3H, ArH), 5.43 (t, $J = 7.4$ Hz, 1H, H-2), 5.33 (t, $J = 7.4$ Hz, 1H, H-3), 5.09 (d, $J = 7.3$ Hz, 1H, H-1), 4.45 (dd, $J = 12.0$, 4.4 Hz, 1H, H-5), 4.00 (td, $J = 7.6$, 4.4 Hz, 1H, H-4), 3.61 (dd, $J = 12.0$, 7.6 Hz, 1H, H-5). **¹³C-NMR** (101 MHz, CDCl₃) δ 167.1 (Bz), 165.2 (Bz), 133.8, 133.6, 133.0, 132.8, 130.2, 130.0, 129.3, 129.2, 128.9, 128.7, 128.6, 128.3, 86.8 (C-1), 76.0 (C-3), 70.2 (C-2), 68.4 (C-4), 67.6 (C-5). **Elemental Analysis:** calc. C: 66.65 H: 4.92 S: 7.12; found: C: 66.64 H: 4.89 S: 6.99



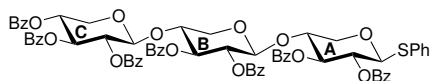
(12) **Phenylsulfenyl chloride** Into a three-neck, round-bottom flask (1 L), fitted with an argon inlet, a pressure-equalizing dropping funnel (500 ml), and a magnetic stir bar, was charged with thiophenol (84 ml), dry triethylamine (1 ml), and dry pentane (400 ml) under a blanket of argon. The remaining neck of the flask was stoppered and the argon was allowed to sweep gently through the flask and out of the pressure-equalizing dropping funnel. The flask and its contents were cooled to 0° C. with an ice bath and stirring was begun. The dropping funnel was charged with sulfuryl chloride (76 ml). The sulfuryl chloride was added dropwise over a 1-hr period to the chilled thiophenol solution with stirring. During this addition, a thick layer of white solid formed. It gradually dissolved as it was broken apart. After the addition was complete, the ice bath was removed and the mixture was allowed to stir for 1 h longer while slowly warming to room temperature. During the course of the addition and subsequent stirring, the clear, pale-yellow solution became dark orange-red. The dropping funnel was replaced with an outlet adapter connected to a vacuum pump and the argon inlet was exchanged for a ground glass stopper. The pentane and excess sulfuryl chloride were removed under reduced pressure at room temperature. After this, the outlet adapter was replaced by a short-path distillation apparatus adapted for use under reduced pressure. The oily red residue was distilled to give phenylsulfenyl chloride as a blood-red liquid (26 g, 87%), by 41-42° C. (1.5 mm). This compound was stored under argon until used.²⁰¹ **¹H-NMR** (400 MHz, CDCl₃) δ 7.70 – 7.65 (m, 2H), 7.46 – 7.39 (m, 3H). **¹³C-NMR** (101 MHz, CDCl₃) δ 135.7, 131.9, 130.2, 129.5.



(13) Phenyl 2,3,4-tri-*O*-benzoyl- β -D-xylopyranosyl-(1 \rightarrow 4)-2,3-*O*-benzoyl-1-thio- β -D-xylopyranoside This compound was obtained

according to General Procedure II in a yield of 79%

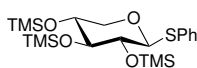
after purification by column chromatography (R_f = 0.18 (3 ethyl acetate / 7 heptane)) $[\alpha]_D^{20}$ = -22 (c 1.0, CHCl_3), $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.02 – 7.91 (m, 11H), 7.56 – 7.50 (m, 5H), 7.45 – 7.38 (m, 9H), 7.36 – 7.33 (m, 3H), 7.28 – 7.27 (m, 2H), 5.67 (t, J = 7.9 Hz, 1H, A-3), 5.63 (t, J = 6.6 Hz, 1H, B-3), 5.34 (t, J = 8.0 Hz, 1H, A-2), 5.24 (dd, J = 6.5, 5.0 Hz, 1H, B-2), 5.06 – 5.01 (m, 1H, B-4), 5.02 (d, J = 8.1 Hz, 1H, A-1), 4.95 (d, J = 4.8 Hz, 1H, B-1), 4.27 (dd, J = 12.2, 4.7 Hz, 1H, A-5a), 4.09 (td, J = 8.3, 4.9 Hz, 1H, A-4), 4.03 (dd, J = 12.4, 3.9 Hz, 1H, B-5a), 3.55 (dd, J = 12.1, 8.6 Hz, 1H, A-5b), 3.43 (dd, J = 12.4, 6.3 Hz, 1H, B-5b). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 165.6 (Bz), 165.5 (Bz), 165.4 (Bz), 165.4 (Bz), 165.2 (Bz), 133.6, 133.43, 132.68, 130.08, 130.06, 130.01, 129.94, 129.90, 129.55, 129.42, 129.32, 129.13, 128.58, 128.54, 128.20, 99.8 (CH, B-1), 86.8 (CH, A-1), 75.2 (CH, A-4), 73.1 (CH, A-3), 70.6 (CH, A-2), 70.2 (CH, B-2), 69.7 (CH, B-3), 68.6 (CH, B-4), 65.6 (CH_2 , A-5), 60.9 (CH_2 , B-5). **HRMS (MALDI)** m/z calcd for $\text{C}_{51}\text{H}_{42}\text{O}_{13}\text{S}$ ($\text{M}+\text{Na}^+$) 917.2238, found 917.2258.



(14) Phenyl 2,3,4-tri-*O*-benzoyl- β -D-xylopyranosyl-(1 \rightarrow 4)-2,3-*O*-benzoyl- β -D-xylopyranosyl-(1 \rightarrow 4)-2,3-*O*-benzoyl- β -1-thio- β -D-xylopyranoside This compound has been obtained in 69% yield applying

General Procedure II in a one-pot fashion. $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.00 – 7.90 (m, 15H), 7.58 – 7.46 (m, 7H), 7.43 – 7.27 (m, 18H), 5.64 – 5.58 (m, 2H, 2xCH, A-3, C-3), 5.54 (t, J = 8.1 Hz, 1H, CH, B-3), 5.36 – 5.26 (m, 1H, CH, A-2), 5.23 – 5.13 (m, 2H, 2xCH, B-2, C-2), 5.03 (m, 1H, CH, C-4), 4.98 (d, J = 8.1 Hz, 1H, CH, A-1), 4.75 (d, J = 6.5 Hz, 1H, CH, B-1), 4.73 (d, J = 5.0 Hz, 1H, CH, C-1), 4.15 (dd, J = 12.0, 4.7 Hz, 1H, CH_2 , A-5a), 4.05 – 3.95 (m, 2H, CH, A-4, CH_2 , C-5a), 3.84 (td, J = 8.2, 4.9 Hz, 1H, CH, B-4), 3.57 (dd, J = 12.3, 4.7 Hz, 1H, B-5a), 3.48 (dd, J = 12.0, 8.7 Hz, 1H, A-5b), 3.37 (dd, J = 12.3, 6.3 Hz, 1H, C-5b), 3.17 (dd, J = 12.2, 8.6 Hz, 1H, B-5b). $^{13}\text{C-NMR}$ (101

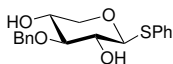
MHz, CDCl_3) δ 165.5 (Bz), 165.5 (Bz), 165.4 (Bz), 165.3 (Bz), 165.3 (Bz), 165.2 (Bz), 165.0 (Bz), 133.5, 133.5, 133.4, 133.3, 133.2, 132.8, 132.6, 130.1, 130.0, 130.0, 129.9, 129.9, 129.8, 129.6, 129.5, 129.5, 129.3, 129.2, 129.1, 129.0, 128.6, 128.5, 128.5, 128.5, 128.4, 128.1, 101.1 (CH, B-1), 99.5 (CH, C-1), 86.7 (CH, A-1), 75.8 (CH, A-4), 74.9 (CH, B-4), 73.1 (CH, A-3), 72.1 (CH, B-3), 71.5 (CH, B-2), 70.4 (CH, A-2), 70.1 (CH, A-2), 69.7 (CH, B-3), 68.6 (CH, C-4), 65.8 (CH_2 , A-5), 62.2 (CH_2 , B-5), 60.9 (CH_2 , C-5). **HRMS (MALDI)** m/z calcd for $\text{C}_{70}\text{H}_{58}\text{O}_{19}\text{S}$ ($\text{M}+\text{Na}^+$) 1257.3185, found 1257.3192



(15) Phenyl 2,3,4-tri-*O*-trimethylsilyl-1-thio- β -

D-xylopyranoside To a mixture of the triol **1** (1 g, 4.13 mmol) and triethylamine (3.65 ml, 26.0 mmol) in anhydrous CH_2Cl_2 (10.5 ml) was added

chlorotrimethylsilane (2.20 ml, 17.33 mmol) at 0°C under argon atmosphere. The reaction was gradually warmed up to room temperature and kept stirring over night. TLC showed full consumption of starting material. The solvent was evaporated under reduced pressure, the residue was diluted with hexane (50 ml), and the resulting mixture was filtered through Celite. The filtrate was concentrated in vacuo, and the residue was purified by flash column chromatography (5% ethylacetate in pentane) to provide **15** (1.77 g, 3.86 mmol, 93% yield) as a colorless oil.²²⁰ $^1\text{H-NMR}$ (300 MHz, CDCl_3) δ 7.49 – 7.45 (m, 2H), 7.32 – 7.28 (m, 2H), 7.27 – 7.22 (m, 1H), 4.66 (d, $J = 8.0$ Hz, 1H, H-1), 3.99 (dd, $J = 11.4$, 4.8 Hz, 1H, H-5a), 3.63 – 3.55 (m, 1H, H-4), 3.53 – 3.40 (m, 2H, H-2, H-3), 3.20 (dd, $J = 11.4$, 9.2 Hz, 1H, H-5b), 0.25 – 0.24 (m, 9H, $3\times\text{CH}_3$), 0.19 – 0.17 (m, 9H, $3\times\text{CH}_3$), 0.16 – 0.14 (m, 9H, $3\times\text{CH}_3$).

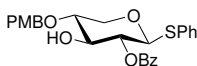


(16) Phenyl 3-*O*-benzyl-1-thio- β -D-xylopyrano-

side This compound was prepared according to a literature procedure, but was only isolated in trace amounts.¹⁴⁷ **¹H-**

NMR (400 MHz, CDCl₃) δ 7.53 – 7.51 (m, 2H), 7.38 –

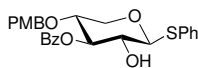
7.28 (m, 8H), 4.69 (d, J = 11.8 Hz, 1H), 4.64 (d, J = 11.8 Hz, 1H), 4.56 (d, J = 9.0 Hz, 1H), 4.10 (dd, J = 11.5, 4.8 Hz, 1H), 3.69 (td, J = 8.4, 2.5 Hz, 1H), 3.49 (ddd, J = 9.6, 8.5, 4.8 Hz, 1H), 3.43 – 3.38 (m, 1H), 3.30 (dd, J = 11.5, 9.7 Hz, 1H), 2.70 (d, J = 3.0 Hz, 1H), 2.68 (d, J = 2.5 Hz, 1H). **¹³C-NMR** (101 MHz, CDCl₃) δ 138.0, 132.9, 132.1, 129.2, 128.8, 128.3, 128.3, 128.0, 88.9, 76.9, 76.6, 73.2, 72.1, 67.1.



(18) Phenyl 2-*O*-benzoyl-4-*O*-*p*-methoxybenzyl-1-thio- β -D-xylopyranoside

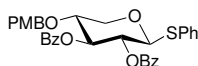
An aqueous 1M solution of sodium hydroxide (7.00 ml) was added with vigorous stirring at -5°C to a solution of diol **67**

(1g, 2.76 mmol), tetrabutylammonium hydrogen sulfate (0.187 g, 0.552 mmol) and benzoyl chloride (0.43 ml, 3.72 mmol) in CH₂Cl₂ (50.0 ml). The mixture was stirred for 30 min. (TLC indicated traces of disubstituted product and traces of starting material). The organic layer was separated, washed with water, dried and concentrated. Column chromatography (1.5 ethyl acetate / 8.5 hexane) afforded the benzyl ester **18** (877 mg, 1.88 mmol, 68%). R_f = 0.23 (3 ethyl acetate / 7 heptane) $[\alpha] = -12.0$ (c 1.0, CHCl₃), **¹H-NMR** (400 MHz, CDCl₃) δ 8.11 – 8.09 (m, 2H), 7.62 – 7.59 (m, 1H), 7.49 – 7.49(m, 4H), 7.30 – 7.28 (m, 5H), 6.90 – 6.88 (m, 2H), 5.09 – 5.05 (m, 1H), 4.80 (d, J = 9.6 Hz, 1H), 4.66 (d, J = 11.5 Hz, 1H), 4.61 (d, J = 11.5 Hz, 1H), 4.12 (dd, J = 11.5, 5.0 Hz, 1H), 3.89 – 3.85 (m, 1H), 3.82 (s, 3H), 3.60 (ddd, J = 9.8, 8.8, 5.1 Hz, 1H), 3.32 (dd, J = 11.4, 10.2 Hz, 1H). **¹³C-NMR** (101 MHz, CDCl₃) δ 166.1, 159.6, 133.5, 132.7, 130.1, 130.0, 129.8, 129.7, 129.1, 128.6, 128.1, 114.2, 114.2, 86.8, 77.1, 75.9, 73.1, 73.0, 67.6, 55.4. **Elemental Analysis:** calc. C: 66.94 H: 5.62 S: 6.87; found: C: 66.98 H: 5.59 S: 6.74



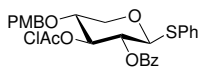
(19) Phenyl 3-*O*-benzoyl-4-*O*-*p*-methoxybenzyl-1-thio- β -D-xylopyranoside The compound is prepared according to the same procedure as compound **18** and obtained in 13% yield. $R_f = 0.16$ (3

ethyl acetate / 7 heptane) $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.14 – 8.07 (m, 2H, ArH), 7.60 – 7.52 (m, 1H, ArH), 7.52 – 7.40 (m, 4H, ArH), 7.31 – 7.13 (m, 5H, ArH), 6.81 – 6.74 (m, 2H, ArH), 5.31 (t, $J = 5.6$ Hz, 1H, H-3), 5.08 (d, $J = 4.9$ Hz, 1H, H-1), 4.58 (d, $J = 11.7$ Hz, 1H, OCH_2Ph), 4.55 (d, $J = 11.7$ Hz, 1H, OCH_2Ph), 4.37 (dd, $J = 11.5, 2.2$ Hz, 1H, H-5), 3.80 (t, $J = 5.2$ Hz, 1H, H-2), 3.74 (s, 3H, OCH_3), 3.67 – 3.56 (m, 2H, H-4, H-5). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 165.9 (Bz), 159.7, 134.2, 133.6, 132.2, 130.2, 129.7, 129.5, 129.3, 129.2, 128.6, 127.9, 127.6, 114.1, 89.2 (C-1), 73.1 (C-4), 72.0 (OCH_2Ph), 71.6 (C-3), 70.4 (C-2), 62.7 (C-5), 55.4 (OCH_3).



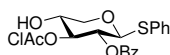
(20) Phenyl 2,3-di-*O*-benzoyl-4-*O*-*p*-methoxybenzyl-1-thio- β -D-xylopyranoside

The diol **10** (0.680 g, 1.88 mmol) was dissolved in pyridine (5 ml) and BzCl (0.436 ml, 3.75 mmol) was added. The reaction mixture was stirred at 22 °C for 2 h, then it was diluted with dichloromethane and washed with 1 M HCl (2x20 ml) and water (2x20 ml). The organic phase was dried over Na_2SO_4 , filtered and the solvent removed under vacuum. The residue was purified by column chromatography to afford the diester **20** (0.990, 84%). $[\alpha]_D^{20} = +55.0$ (c 1.00, CHCl_3). $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 7.99 – 7.95 (m, 4H), 7.56 – 7.51 (m, 2H), 7.50 – 7.46 (m, 2H), 7.42 – 7.34 (m, 4H), 7.31 – 7.27 (m, 3H), 7.15 – 7.12 (m, 2H), 6.74 – 6.71 (m, 2H), 5.60 (t, $J = 8.0$ Hz, 1H, H-3), 5.34 (t, $J = 8.0$ Hz, 1H, H-2), 5.04 (d, $J = 8.1$ Hz, 1H, H-1), 4.55 (d, $J = 11.8$ Hz, 1H, OCH_2Ph), 4.52 (d, $J = 11.8$ Hz, 1H, OCH_2Ph), 4.28 (dd, $J = 11.9, 4.6$ Hz, 1H, H-5), 3.79 – 3.74 (m, 1H, H-4), 3.75 (s, 3H, OCH_3), 3.57 (dd, $J = 11.9, 8.5$ Hz, 1H, H-5). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 165.7 (Bz), 165.4 (Bz), 159.5, 133.4, 133.2, 132.5, 130.1, 130.0, 129.7, 129.7, 129.5, 129.4, 129.1, 128.5, 128.5, 128.1, 113.9, 86.9 (C-1), 73.9 (C-3), 73.6 (C-4), 72.5 (OCH_2Ph), 70.6 (C-2), 66.4 (H-5), 55.3 (OCH_3). **HRMS (MALDI)** m/z calcd for $\text{C}_{33}\text{H}_{30}\text{O}_7\text{S}$ ($\text{M}+\text{Na}^+$) 593.1604, found 593.1617.



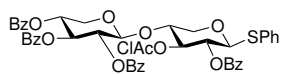
(21) Phenyl 2-*O*-benzoyl-3-*O*-chloroacetyl-4-*O*-*p*-methoxybenzyl-1-thio- β -D-xylopyranoside Compound **18** (500 mg, 1.07 mmol) was dissolved

in CH_2Cl_2 (10.70 ml) and pyridine (0.58 ml, 7.18 mmol), and thereafter, chloroacetyl chloride (0.17 ml, 2.14 mmol) was added to the solution while cooling on ice. The obtained mixture was stirred at room temperature for 4 hours. After completion of the reaction, the reaction solution was extracted with CH_2Cl_2 , washed with HCl, and then dehydrated with MgSO_4 , followed by vacuum concentration. The resultant was purified by silica gel column chromatography (toluene : ethyl acetate = 15 : 1), so as to obtain compound **21** (440 mg, 0.810 mmol, 76% yield). $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.02 – 7.99 (m, 2H), 7.61 – 7.56 (m, 1H), 7.46 – 7.40 (m, 4H), 7.30 – 7.26 (m, 3H), 7.22 – 7.18 (m, 2H), 6.90 – 6.85 (m, 2H), 5.35 (t, J = 8.8 Hz, 1H), 5.14 (t, J = 9.1 Hz, 1H), 4.84 (d, J = 9.3 Hz, 1H), 4.55 (d, J = 11.8 Hz, 1H), 4.50 (d, J = 11.8 Hz, 1H), 4.15 (dd, J = 6.8, 5.0 Hz, 1H), 3.87 (d, J = 14.7 Hz, 1H), 3.84 (d, J = 14.7 Hz, 1H), 3.81 (s, 3H), 3.69 (td, J = 9.6, 5.1 Hz, 1H), 3.41 (dd, J = 11.7, 9.8 Hz, 1H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 166.7, 165.4, 159.7, 133.6, 132.8, 132.4, 130.1, 129.7, 129.7, 129.2, 129.1, 128.7, 128.3, 114.1, 87.0, 76.4, 74.3, 72.9, 70.6, 67.5, 55.4, 40.6. **HRMS (MALDI)** m/z calcd for $\text{C}_{28}\text{H}_{27}\text{ClO}_7\text{S}$ ($\text{M}+\text{Na}^+$) 566.1136, found 566.1145.



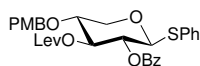
(22) Phenyl 2-*O*-benzoyl-3-*O*-chloroacetyl-1-thio- β -D-xylopyranose This compound was prepared according to General Procedure IV in 89% yield.

$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.06 – 7.98 (m, 3H), 7.62 – 7.57 (m, 1H), 7.49 – 7.43 (m, 6H), 7.33 – 7.26 (m, 3H), 5.25 – 5.18 (m, 2H), 4.96 – 4.93 (m, 1H), 4.30 (dd, J = 11.8, 4.9 Hz, 1H), 4.05 (d, J = 14.9 Hz, 1H), 3.99 (d, J = 14.9 Hz, 1H), 3.93 (td, J = 8.6, 5.0 Hz, 2H), 3.49 (dd, J = 11.8, 8.9 Hz, 1H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 167.5, 165.3, 133.8, 132.9, 132.5, 130.0, 129.2, 128.7, 128.6, 128.4, 126.4, 86.9, 77.4, 70.2, 68.3, 68.1, 40.7.



(23) Phenyl 2,3,4-tri-*O*-benzoyl- β -D-xylopyranosyl-(1 \rightarrow 4)-2-*O*-benzoyl-3-*O*-chloroacetyl-1-thio- β -D-xylopyranoside

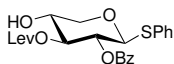
This compound was prepared according to General Procedure II in 42% yield. $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.08 – 7.98 (m, 4H), 7.98 – 7.94 (m, 4H), 7.64 – 7.46 (m, 7H), 7.44 – 7.36 (m, 9H), 7.33 – 7.26 (m, 1H), 5.75 (t, J = 7.3 Hz, 1H, H-3'), 5.45 (t, J = 8.8 Hz, 1H, H-3), 5.30 (dd, J = 7.3, 5.4 Hz, 1H, H-2'), 5.27 (dd, J = 7.1, 4.4 Hz, 1H, H-4'), 5.20 (t, J = 9.1 Hz, 1H, H-2), 4.91 (d, J = 5.4 Hz, 1H, H-1'), 4.83 (d, J = 9.3 Hz, 1H, H-1), 4.40 (dd, J = 12.2, 4.3 Hz, 1H, H-5a'), 4.18 – 4.09 (m, 1H, H-5a), 4.06 – 3.96 (m, 3H, CH_2Cl , H-4), 3.71 (dd, J = 12.2, 7.0 Hz, 1H, H-5b'), 3.40 (dd, J = 11.8, 9.8 Hz, 1H, H-5b). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ = 166.7 (ClAc), 165.6 (Bz), 165.5 (Bz), 165.4 (Bz), 165.1 (Bz), 133.7, 133.6, 133.6, 133.3, 133.2, 131.9, 130.1, 130.0, 130.0, 129.9, 129.8, 129.2, 129.2, 129.1, 129.1, 129.0, 128.7, 128.6, 128.6, 128.5, 100.5 (H-1'), 86.7 (H-1), 75.7 (C-4), 75.6 (C-3), 70.6 (C-2'), 70.4 (C-2), 70.2 (C-3'), 69.0 (H-4'), 66.9 (C-5), 61.8 (C-5'), 40.8 (CH_2Cl).



(24) Phenyl 2-*O*-benzoyl-3-*O*-levulinoyl-4-*O*-*p*-methoxybenzyl-1-thio- β -D-xylopyranoside

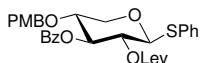
A solution of DCC (4.56 g, 22.08 mmol) and DMAP (0.135 g, 1.104 mmol) in CH_2Cl_2 (5.51 ml) was added to a solution of **18** (5.15 g, 11.04 mmol) and 4-oxopentanoic acid (1.92 g, 16.56 mmol) in CH_2Cl_2 (55.10 ml) at 0°C. After stirring for 40 min at ambient temperature, the mixture was filtered and the filtrate was concentrated in vacuo. Purification by column chromatography (3 ethyl acetate / 7 heptane) afforded 6.18 g of product (10.94 mmol, 99 %). R_f = 0.21 (3 ethyl acetate / 7 heptane) $[\alpha] = +15$ (c 1.0, CHCl_3), $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.02 – 7.99 (m, 2H), 7.59 – 7.55 (m, 1H), 7.44 – 7.40 (m, 4H), 7.29 – 7.27 (m, 2H), 7.25 – 7.22 (m, 2H), 6.88 – 6.85 (m, 2H), 5.34 – 5.30 (m, 1H), 5.17 – 5.12 (m, 1H), 4.90 (d, J = 8.5 Hz, 1H), 4.58 (d, J = 11.7 Hz, 1H), 4.53 (d, J = 11.7 Hz, 1H), 4.14 (dd, J = 11.8, 4.8 Hz, 1H), 3.80 (s, 3H), 3.68 – 3.62 (m, 1H), 3.44 (dd, J = 11.8, 9.0 Hz, 1H), 2.66 – 2.50 (m, 3H), 2.46 – 2.39 (m, 1H), 2.06 (s, 3H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 206.1, 171.9, 165.4, 159.6, 133.5, 133.0, 132.5, 130.1, 129.9, 129.7, 129.5, 129.1, 128.6, 128.1, 114.0, 86.9, 74.0, 72.8, 70.7, 66.9, 55.4, 38.0, 29.8, 28.2. **HRMS (MALDI)** m/z calcd for $\text{C}_{31}\text{H}_{32}\text{O}_8\text{S}$ ($\text{M}+\text{Na}^+$) 587.1710,

found 587.1722.



(25) Phenyl 2-*O*-benzoyl-3-*O*-levulinoyl-1-thio- β -D-xylopyranoside This compound has been prepared, starting from compound **24** according to General Procedure IV in 83% yield. $[\alpha] = 0$ (c 1.0,

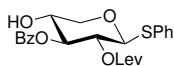
CHCl_3), $R_f = 0.13$ (4.5 ethyl acetate/5.5 heptane) $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.04 – 8.01 (m, 2H), 7.61 – 7.57 (m, 1H), 7.47 – 7.44 (m, 4H), 7.29 – 7.28 (m, 3H), 5.23 – 5.16 (m, 2H), 4.94 – 4.91 (m, 1H), 4.29 (dd, $J = 11.8$, 4.9 Hz, 1H), 3.94 – 3.89 (m, 1H), 3.49 (dd, $J = 11.8$, 9.1 Hz, 1H), 2.80 – 2.63 (m, 2H), 2.54 (ddd, $J = 13.4$, 8.3, 5.1 Hz, 1H), 2.40 (ddd, $J = 16.7$, 6.4, 5.5 Hz, 1H), 2.10 (s, 3H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 207.7, 172.8, 165.3, 133.6, 132.9, 132.7, 130.1, 129.4, 129.1, 128.7, 128.2, 87.1, 76.8, 76.4, 70.3, 68.5, 68.3, 38.5, 29.8, 28.4. **HRMS (MALDI)** m/z calcd for $\text{C}_{23}\text{H}_{24}\text{O}_7\text{S}$ ($\text{M}+\text{Na}^+$) 467.1135, found 467.1145.



(26) Phenyl 3-*O*-benzoyl-2-*O*-levulinyl-4-*O*-*p*-methoxybenzyl-1-thio- β -D-xylopyranoside Compound **19** (1.0 g, 2.14 mmol)

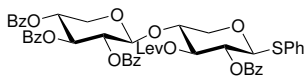
was dissolved in dichloromethane (30 ml) followed by addition of DCC (0.53 g, 2.57 mmol), DMAP (0.261 g, 2.14 mmol) and LevOH (0.33 ml, 3.22 mmol). A white precipitate formed and complete conversion was observed after 40 minutes at 22°C. The reaction mixture was filtered through a Celite pad and the filtrate was concentrated to dryness. The residue was purified by column chromatography (heptane/ethyl acetate, 7:3) to give **26** as a colorless amorphous solid (1.21 g, 98%). $[\alpha]_D^{20} = -13.3$ (c 0.27, CHCl_3). $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.03 - 7.98 (m, 2H, ArH), 7.62 - 7.56 (m, 1H, ArH), 7.52 - 7.42 (m, 4H, ArH), 7.35 - 7.28 (m, 3H, ArH), 7.13 - 7.07 (m, 2H, ArH), 6.74 - 6.68 (m, 2H, ArH), 5.42 (t, $J = 8.4$ Hz, 1H, H-3), 5.04 (t, $J = 8.6$ Hz, 1H, H-2), 4.82 (d, $J = 8.7$ Hz, 1H, H-1), 4.51 (d, $J = 11.9$ Hz, 1H, OCH_2Ph), 4.47 (d, $J = 11.9$ Hz, 1H, OCH_2Ph), 4.17 (dd, $J = 11.8$, 4.9 Hz, 1H, H-5), 3.74 (s, 3H, OCH_3), 3.69 (td, $J = 9.2$, 4.9 Hz, 1H, H-4), 3.44 (dd, $J = 11.8$, 9.2 Hz, 1H, H-5), 2.63 - 2.43 (m, 4H, 2x CH_2), 2.04 (s, 3H, CH_3).

$^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 205.8 (Lev), 171.4 (Lev), 165.6 (Bz), 159.4, 133.3, 132.7, 132.5, 130.0, 129.6, 129.5, 129.0, 128.4, 128.1, 113.8, 86.6 (C-1), 74.2 (C-3), 73.9 (C-4), 72.4 (OCH_2Ph), 70.3 (C-2), 66.9 (C-5), 55.2 (OCH_3), 37.8 (CH_2), 29.6 (CH_2), 28.0 (CH_3). **HRMS (MALDI)** m/z calcd for $\text{C}_{31}\text{H}_{32}\text{O}_8\text{S}$ ($\text{M}+\text{Na}^+$) 587.1710, found 587.2781.



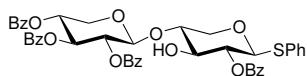
(27) Phenyl 3-*O*-benzoyl-2-*O*-levulinoyl-1-thio- β -D-xylopyranoside This compound was prepared according to General Procedure IV in 90% yield. $[\alpha]_D^{20} = +25.6$ (c 0.70, CHCl_3).

$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.23 – 7.89 (m, 2H, ArH), 7.70 – 7.28 (m, 8H, ArH), 5.20 – 5.13 (m, 2H, H-2, H-3), 4.96 – 4.90 (m, 1H, H-1), 4.38 (dd, $J = 11.9, 4.4$ Hz, 1H, H-5), 3.95 – 3.87 (m, 1H, H-4), 3.54 (dd, $J = 11.9, 7.9$ Hz, 1H, H-5), 2.72 – 2.65 (m, 2H, CH_2), 2.65 – 2.50 (m, 2H, CH_2), 2.09 (s, 3H, CH_3). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 205.9 (Lev), 171.2 (Lev), 166.9 (Bz), 133.7, 132.9, 132.5, 130.2, 129.1, 128.9, 128.6, 128.1, 86.4 (C-1), 75.7 (C-3), 69.7 (C-2), 68.2 (C-4), 67.3 (C-5), 37.9 (CH_2), 29.6 (CH_2), 28.0 (CH_3). **HRMS (MALDI)** m/z calcd for $\text{C}_{23}\text{H}_{24}\text{O}_7\text{S}$ ($\text{M}+\text{Na}^+$) 467.1134, found 467.1146.



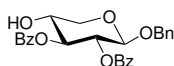
(28) Phenyl 2,3,4-tri-*O*-benzoyl- β -D-xylopyranosyl-(1 \rightarrow 4)-2-*O*-benzoyl-3-*O*-levulinoyl-1-thio- β -D-xylopyranoside This compound was prepared according to General Procedure II in 86% yield. Purification was accomplished by flash chromatography ($R_f = 0.20$ (4 ethyl acetate / 6 heptane)). $[\alpha]_D^{20} = -41.4$ (c 1.0, CHCl_3), $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.05 – 8.00 (m, 4H), 7.98 – 7.92 (m, 4H), 7.63 – 7.45 (m, 6H), 7.42 – 7.32 (m, 9H), 7.28 – 7.27 (m, 2H), 5.70 (t, $J = 6.5$ Hz, 1H), 5.40 (t, $J = 8.3$ Hz, 1H), 5.31 – 5.21 (m, 2H), 5.16 (t, $J = 8.4$ Hz, 1H), 4.94 (d, $J = 4.7$ Hz, 1H, H-1'), 4.89 (d, $J = 8.6$ Hz, 1H, H-1), 4.48 (dd, $J = 12.4, 3.8$ Hz, 1H), 4.19 (dd, $J = 12.0, 4.9$ Hz, 1H), 3.99 (td, $J = 8.7, 5.0$ Hz, 1H), 3.75 (dd, $J = 12.4, 6.1$ Hz, 1H), 3.44 (dd, $J = 12.0, 9.1$ Hz, 1H), 2.67 – 2.50 (m, 3H), 2.51 – 2.41 (m, 1H), 1.97 (s, 3H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 205.9, 171.5, 165.7, 165.5, 165.4, 165.2, 133.6, 133.5, 132.9, 132.7, 132.5, 130.3, 130.1, 130.0, 129.9, 129.6, 129.3,

129.2, 129.2, 129.1, 129.1, 128.7, 128.6, 128.6, 128.6, 128.3, 99.4, 86.7, 74.6, 73.2, 70.7, 70.2, 69.6, 68.7, 66.1, 61.1, 37.9, 29.6, 28.1. **Elemental Analysis:** calc. C: 66.21 H: 4.99 S: 3.61; found: C: 66.01 H: 5.00 S: 3.59



(29) Phenyl 2,3,4-tri-*O*-benzoyl- β -D-xylopyranosyl-(1 \rightarrow 4)-2-*O*-benzoyl-1-thio- β -D-xylopyranoside This compound was prepared according to General Procedure V in 90% yield.

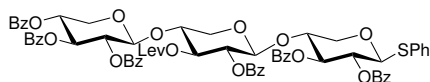
$[\alpha]_D^{20} = -24$ (c 1.0, CHCl_3), $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.12 – 8.10 (m, 2H), 7.97 – 7.92 (m, 6H), 7.62 – 7.52 (m, 3H), 7.50 – 7.46 (m, 3H), 7.41 – 7.32 (m, 8H), 7.27 – 7.26 (m, 3H), 5.86 – 5.81 (m, 1H), 5.43 (dd, $J = 8.7, 7.1$ Hz, 1H), 5.40 – 5.34 (m, 1H), 5.15 – 5.10 (m, 1H), 4.86 (d, $J = 6.9$ Hz, 1H), 4.74 (d, $J = 9.9$ Hz, 1H), 4.46 (dd, $J = 11.8, 5.0$ Hz, 1H), 3.95 – 3.86 (m, 2H), 3.82 – 3.76 (m, 1H), 3.74 (bs, 1H), 3.68 (dd, $J = 11.7, 9.2$ Hz, 1H), 3.32 – 3.27 (m, 1H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 165.7, 165.6, 165.6, 165.2, 133.7, 133.5, 133.4, 133.0, 132.3, 130.1, 130.0, 129.9, 129.8, 129.0, 129.0, 129.0, 128.7, 128.6, 128.5, 128.2, 101.7, 86.7, 80.4, 75.1, 72.2, 71.3, 71.1, 69.3, 67.1, 62.7. **HRMS (MALDI)** m/z calcd for $\text{C}_{44}\text{H}_{38}\text{O}_{12}\text{S}$ ($\text{M}+\text{Na}^+$) 813.1976, found 813.1992



(30) Benzyl 2,3-di-*O*-benzoyl- β -D-xylopyranoside Starting material **20** (200 mg, 0.350 mmol) and the acceptor, benzyl alcohol (0.044 ml, 0.420 mmol), were mixed in the reaction flask and dried overnight

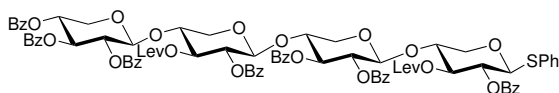
on a vacuum line. The reactants were dissolved in dichloromethane (6 ml) and cooled to -40°C , NIS (94 mg, 0.420 mmol) and triflic acid (9 μl , 0.105 mmol) were added to the stirring mixture. Full conversion of the starting material was observed via TLC analysis after 3 hours and the reaction was neutralized with Et_3N (0.145 ml, 1.05 mmol). The resulting mixture was stirred with 6 ml of 1 M $\text{Na}_2\text{S}_2\text{O}_3$ until the yellow color disappeared. The organic phase was diluted with dichloromethane and washed with brine, dried over Na_2SO_4 , filtered, concentrated and purified with flash chromatography (6 heptane : 4 ethyl acetate) yielding **30** (0.155 g, 65%) as a white solid. The analytical data are in accordance with the literature.²²¹ $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 7.99 – 7.94 (m, 4H, ArH), 7.59 – 7.19 (m, 11H, ArH), 5.43 (dd, $J = 7.8, 6.0$ Hz, 1H,

H-2), 5.26 (t, $J = 7.6$ Hz, 1H, H-3), 4.89 (d, $J = 12.2$ Hz, 1H, OCH₂Ph), 4.78 (d, $J = 6.0$ Hz, 1H, H-1), 4.65 (d, $J = 12.2$ Hz, 1H, OCH₂Ph), 4.26 (dd, $J = 12.0, 4.5$ Hz, 1H, H-5), 4.01 (td, $J = 7.6, 4.5$ Hz, 1H, H-4), 3.54 (dd, $J = 12.0, 7.8$ Hz, 1H, H-5). ¹³C-NMR (101 MHz, CDCl₃) δ 167.2 (Bz), 165.3 (Bz), 137.0, 133.7, 133.5, 130.1, 130.0, 129.4, 129.0, 128.6, 128.5, 128.5, 128.0, 99.1 (C-1), 75.4 (C-3), 70.5 (C-2), 70.4 (OCH₂Ph), 68.7 (C-4), 64.6 (C-5).



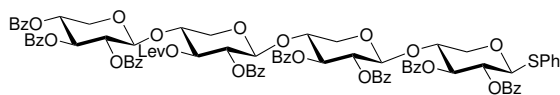
(31) Phenyl 2,3,4-tri-*O*-benzoyl-β-D-xylopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-levulinoyl-β-D-xylopyranosyl-(1→4)-2,3-di-*O*-benzoyl-β-1-thio-β-D-xylopyranoside

This compound was prepared according to general procedure I in 88% yield. $R_f = 0.22$ (4 acetone / 6 heptane) $[\alpha] = -24$ (c 1.0, CHCl₃), ¹H-NMR (400 MHz, CDCl₃) δ 7.93 – 7.83 (m, 13H), 7.53 – 7.21 (m, 22H), 5.58 (t, $J = 6.6$ Hz, 1H), 5.51 (t, $J = 7.9$ Hz, 1H), 5.23 – 5.18 (m, 2H), 5.17 – 5.13 (m, 1H), 5.09 (dd, $J = 6.4, 4.9$ Hz, 1H), 4.94 (dd, $J = 8.2, 6.6$ Hz, 1H), 4.89 (d, $J = 8.1$ Hz, 1H), 4.63 (d, $J = 4.7$ Hz, 1H), 4.58 (d, $J = 6.4$ Hz, 1H), 4.33 (dd, $J = 12.4, 3.8$ Hz, 1H), 4.06 (dd, $J = 12.0, 4.7$ Hz, 1H), 3.88 (td, $J = 8.2, 4.9$ Hz, 1H), 3.66–3.57 (m, 2H), 3.44 (dd, $J = 12.2, 4.8$ Hz, 1H), 3.38 (dd, $J = 12.0, 8.6$ Hz, 1H), 3.01 (dd, $J = 12.2, 8.7$ Hz, 1H), 2.57 – 2.31 (m, 4H), 1.89 (s, 3H). ¹³C-NMR (101 MHz, CDCl₃) δ 205.9, 171.9, 165.6, 165.5, 165.4, 165.3, 165.2, 165.1, 133.6, 133.5, 133.5, 133.4, 133.3, 132.9, 132.6, 130.1, 130.1, 130.0, 129.9, 129.8, 129.6, 129.5, 129.4, 129.4, 129.2, 129.1, 129.1, 128.7, 128.6, 128.6, 128.5, 128.5, 128.2, 101.0, 99.2, 86.8, 75.6, 74.2, 73.1, 71.9, 71.6, 70.4, 70.2, 69.7, 68.8, 65.9, 62.2, 61.1, 37.8, 29.6, 28.0. **HRMS (MALDI)** m/z calcd for C₆₈H₆₀O₂₀S (M+Na⁺) 1251.3291, found 1251.3308.



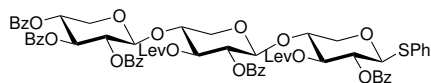
(34) Phenyl 2,3,4-tri-*O*-benzoyl- β -D-xylopyranosyl-(1 \rightarrow 4)-2-*O*-benzoyl-3-*O*-levulinoyl- β -D-xylo-

pyranosyl-(1 \rightarrow 4)-2,3-*O*-benzoyl- β -D-xylopyranosyl-(1 \rightarrow 4)-2-*O*-benzoyl-3-*O*-levulinoyl- β -1-thio- β -D-xylopyranoside This compound was synthesized according to General Procedure II in 67% yield. $R_f = 0.16$ (4 acetone / 6 heptane) $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.02 – 7.87 (m, 15H), 7.61 – 7.46 (m, 6H), 7.47 – 7.41 (m, 5H), 7.40 – 7.34 (m, 10H), 7.35 – 7.26 (m, 2H), 7.25 – 7.23 (m, 2H), 5.65 (t, $J = 6.5$ Hz, 1H), 5.49 (t, $J = 7.9$ Hz, 1H), 5.31 – 5.26 (m, 2H), 5.24 – 5.20 (m, 1H), 5.17 – 5.13 (m, 1H), 5.11 – 5.07 (m, 2H), 5.02 (dd, $J = 8.4, 6.6$ Hz, 1H), 4.82 (d, $J = 8.5$ Hz, 1H), 4.72 (d, $J = 4.6$ Hz, 1H), 4.66 (d, $J = 6.0$ Hz, 1H), 4.66 (d, $J = 6.6$ Hz, 1H), 4.41 (dd, $J = 12.4, 3.7$ Hz, 1H), 4.05 (dd, $J = 12.0, 4.8$ Hz, 1H), 3.98 (dd, $J = 12.2, 4.5$ Hz, 1H), 3.95 – 3.86 (m, 1H), 3.82 (td, $J = 8.6, 5.0$ Hz, 1H), 3.74 (dt, $J = 8.1, 4.1$ Hz, 1H), 3.68 (dd, $J = 12.5, 6.2$ Hz, 1H), 3.55 (dd, $J = 12.2, 4.7$ Hz, 1H), 3.38 (dd, $J = 12.0, 8.0$ Hz, 1H), 3.32 (dd, $J = 11.9, 9.1$ Hz, 1H), 3.11 (dd, $J = 12.2, 8.8$ Hz, 1H), 2.64 – 2.30 (m, 8H), 1.97 (s, 3H), 1.95 (s, 3H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 206.0, 205.9, 171.9, 171.6, 165.6, 165.5, 165.4, 165.3, 165.2, 165.2, 165.1, 133.6, 133.5, 133.5, 133.4, 133.2, 132.8, 132.5, 130.1, 130.1, 130.1, 129.9, 129.8, 129.7, 129.5, 129.4, 129.4, 129.2, 129.1, 129.1, 128.6, 128.6, 128.4, 128.2, 100.8, 100.5, 99.1, 86.6, 77.4, 75.1, 74.9, 74.3, 73.0, 72.0, 71.9, 71.6, 71.1, 70.5, 70.1, 69.6, 68.7, 66.1, 62.5, 62.3, 61.0, 37.8, 37.8, 29.7, 29.7, 28.0, 28.0. **HRMS (MALDI)** m/z calcd for $\text{C}_{85}\text{H}_{78}\text{O}_{27}\text{S}$ ($\text{M}+\text{Na}^+$) 1585.4343, found 1585.4362.



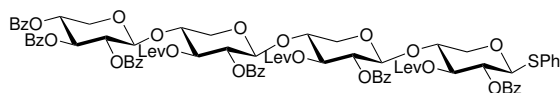
(35) Phenyl 2,3,4-tri-
O-benzoyl- β -D-xylo-
pyranosyl-(1 \rightarrow 4)-2-*O*-
benzoyl-3-*O*-levulinoyl

- β -D-xylopyranosyl-(1 \rightarrow 4)-2-*O*-benzoyl- β -D-xylopyranosyl-(1 \rightarrow 4)-2-*O*-benzoyl- β -1-thio- β -D-xylopyranoside This compound was prepared in 50% yield according to General Procedure II. $R_f = 0.25$ (4.5 acetone / 5.5 heptane) $[\alpha] = -20$ (c 1.0, CHCl_3), $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 7.94 – 7.78 (m, 17H), 7.57 – 7.37 (m, 7H), 7.36 – 7.25 (m, 11H), 7.26 – 7.19 (m, 2H), 7.18 – 7.15 (m, 2H), 5.57 (t, $J = 6.6$ Hz, 1H), 5.50 (t, $J = 7.9$ Hz, 1H), 5.36 (t, $J = 8.2$ Hz, 1H), 5.20 (t, $J = 8.0$ Hz, 1H), 5.18 – 5.00 (m, 4H), 4.88 (d, $J = 8.1$ Hz, 1H), 4.90 – 4.81 (m, 1H), 4.63 – 4.57 (m, 2H), 4.33 (d, $J = 6.5$ Hz, 1H), 4.36 – 4.27 (m, 1H), 4.03 (dd, $J = 12.1, 4.8$ Hz, 1H), 3.86 (td, $J = 8.3, 5.0$ Hz, 1H), 3.72 – 3.65 (m, 1H), 3.66 – 3.53 (m, 3H), 3.41 – 3.30 (m, 2H), 2.99 (dd, $J = 12.1, 8.7$ Hz, 1H), 2.92 (dd, $J = 12.2, 8.6$ Hz, 1H), 2.56 – 2.27 (m, 4H), 1.89 (s, 3H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 205.9, 171.8, 165.6, 165.4, 165.4, 165.3, 165.1, 165.1, 133.5, 133.5, 133.4, 133.2, 132.8, 132.6, 130.1, 130.0, 129.9, 129.9, 129.8, 129.8, 129.6, 129.5, 129.4, 129.2, 129.1, 129.1, 128.6, 128.6, 128.6, 128.5, 128.4, 128.2, 101.1, 100.6, 99.2, 86.7, 75.8, 75.2, 74.3, 73.1, 72.1, 71.9, 71.5, 71.4, 70.4, 70.2, 69.7, 68.8, 62.4, 62.2, 61.1, 53.9, 37.8, 29.4, 28.0. **HRMS (MALDI)** m/z calcd for $\text{C}_{87}\text{H}_{76}\text{O}_{26}\text{S}$ ($\text{M}+\text{Na}^+$) 1591.4238, found 1591.4253.



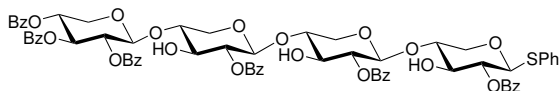
(36) Phenyl 2,3,4-tri-*O*-benzoyl- β -D-xylopyranosyl-(1 \rightarrow 4)-2-*O*-benzoyl-3-*O*-levulinoyl- β -D-xylopyranosyl-(1 \rightarrow 4)-2-*O*-benzoyl-3-*O*-levulinoyl-1-thio- β -D-xylopyranoside

This compound was synthesized according to General Procedure II in 88% yield. ($R_f = 0.35$ (1 ethyl acetate : 1 heptane)). $[\alpha] = -36$ (c 1.0, CHCl_3), $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.02 – 7.92 (m, 11H), 7.61 – 7.51 (m, 5H), 7.49 – 7.43 (m, 4H), 7.40 – 7.32 (m, 8H), 7.25 – 7.24 (m, 2H), 5.70 (t, $J = 6.6$ Hz, 1H), 5.32 (t, $J = 8.1$ Hz, 1H), 5.27 – 5.23 (m, 3H), 5.10 (t, $J = 8.3$ Hz, 1H), 5.02 (dd, $J = 8.3, 6.5$ Hz, 1H), 4.93 (d, $J = 4.8$ Hz, 1H), 4.83 (d, $J = 8.5$ Hz, 1H), 4.64 (d, $J = 6.4$ Hz, 1H), 4.45 (dd, $J = 12.4, 3.9$ Hz, 1H), 4.08 – 4.05 (m, 1H), 4.04 – 4.01 (m, 1H), 3.92 (td, $J = 8.1, 4.9$ Hz, 1H), 3.82 (td, $J = 8.6, 5.0$ Hz, 1H), 3.73 (dd, $J = 12.4, 6.2$ Hz, 1H), 3.39 (dd, $J = 12.1, 8.4$ Hz, 1H), 3.33 (dd, $J = 11.9, 9.0$ Hz, 1H), 2.62 – 2.51 (m, 5H), 2.50 – 2.36 (m, 3H), 2.04 (s, 3H), 1.98 (s, 3H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 206.1, 206.0, 172.0, 171.6, 165.6, 165.4, 165.3, 165.2, 133.5, 132.7, 132.6, 130.1, 130.1, 130.1, 130.0, 130.0, 129.5, 129.4, 129.3, 129.2, 129.1, 128.7, 128.6, 128.2, 100.6, 99.3, 86.6, 74.9, 74.3, 73.0, 71.9, 71.4, 70.5, 70.3, 69.7, 68.8, 66.1, 62.5, 61.2, 37.9, 37.9, 29.8, 29.7, 28.1. **HRMS (MALDI)** m/z calcd for $\text{C}_{66}\text{H}_{62}\text{O}_{21}\text{S}$ ($\text{M}+\text{Na}^+$) 1245.3397, found 1245.3412.



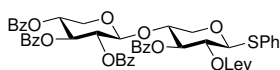
(37) Phenyl 2,3,4-tri-*O*-benzoyl- β -D-xylopyranosyl-(1 \rightarrow 4)-2-*O*-benzoyl-3-*O*-levulinoyl- β -D-xy-

lopyranosyl-(1 \rightarrow 4)-2-*O*-benzoyl-3-*O*-levulinoyl- β -D-xylopyranosyl-(1 \rightarrow 4)-2-*O*-benzoyl-3-*O*-levulinoyl- β -1-thio- β -D-xylopyranoside This compound was prepared according to general procedure II in 69% yield. R_f = 0.27 (1 acetone : 1 heptane), $[\alpha] = -54$ (c 1.0, CHCl_3), $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.01 – 7.92 (m, 13H), 7.59 – 7.51 (m, 5H), 7.49 – 7.42 (m, 6H), 7.40 – 7.32 (m, 8H), 7.24 – 7.22 (m, 3H), 5.69 (t, J = 6.5 Hz, 1H), 5.32 (t, J = 8.2 Hz, 1H), 5.27 – 5.23 (m, 3H), 5.20 (t, J = 8.1 Hz, 1H), 5.08 (t, J = 8.4 Hz, 1H), 5.03 (dd, J = 8.2, 6.8 Hz, 1H), 4.96 (dd, J = 8.0, 6.6 Hz, 1H), 4.92 (d, J = 4.7 Hz, 1H), 4.81 (d, J = 8.4 Hz, 1H), 4.63 (d, J = 6.5 Hz, 1H), 4.56 (d, J = 6.3 Hz, 1H), 4.46 (dd, J = 12.4, 3.7 Hz, 1H), 4.04 – 4.02 (m, 1H), 4.01 – 3.99 (m, 1H), 3.95 – 3.89 (m, 2H), 3.80 – 3.71 (m, 3H), 3.38 (dd, J = 12.0, 8.5 Hz, 1H), 3.32-3.24 (m, 2H), 2.64 – 2.48 (m, 8H), 2.46 – 2.32 (m, 4H), 2.04 (s, 3H), 2.01 (s, 3H), 1.98 (s, 3H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 206.0, 205.9, 205.9, 171.8, 171.6, 171.5, 165.5, 165.3, 165.2, 165.1, 165.0, 133.4, 132.6, 132.4, 123.0, 129.9, 129.8, 129.4, 129.3, 129.1, 129.0, 128.9, 128.5, 128.4, 128.1, 100.3, 100.3, 99.1, 86.4, 74.7, 74.4, 74.2, 72.9, 71.9, 71.6, 71.3, 71.0, 70.3, 70.1, 69.5, 68.6, 65.9, 62.4, 61.0, 37.8, 37.7, 37.7, 29.7, 29.5, 27.9, 27.8. **HRMS (MALDI)** m/z calcd for $\text{C}_{83}\text{H}_{80}\text{O}_{28}\text{S}$ ($\text{M}+\text{Na}^+$) 1579.4449, found 1579.4480.



(38) Phenyl 2,3,4-tri-*O*-benzoyl- β -D-xylopyranosyl-(1 \rightarrow 4)-2-*O*-benzoyl- β -D-xylopyranosyl-(1 \rightarrow 4)-2-

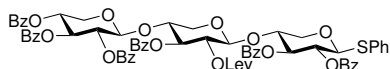
O-benzoyl- β -D-xylopyranosyl-(1 \rightarrow 4)-2-*O*-benzoyl- β -1-thio- β -D-xylopyranoside This compound was prepared according to General Procedure V in 97% yield. $[\alpha] = -38$ (c 1.0, CHCl₃), $^1\text{H-NMR}$ (400 MHz, CHCl₃) δ 8.06 – 7.99 (m, 5H), 7.96 – 7.90 (m, 6H), 7.63 – 7.51 (m, 5H), 7.49 – 7.42 (m, 6H), 7.43 – 7.31 (m, 8H), 7.25 – 7.21 (m, 2H), 7.21 – 7.13 (m, 3H), 5.82 (t, $J = 8.9$ Hz, 1H), 5.42 (dd, $J = 9.0, 7.1$ Hz, 1H), 5.42 – 5.31 (m, 1H), 5.12 – 4.99 (m, 3H), 4.82 (d, $J = 7.0$ Hz, 1H), 4.64 (d, $J = 10.0$ Hz, 1H), 4.50 (d, $J = 7.9$ Hz, 1H), 4.46 (d, $J = 8.0$ Hz, 1H), 4.46 – 4.42 (m, 1H), 3.87 – 3.54 (m, 13H), 3.34 – 3.23 (m, 1H), 3.26 – 3.12 (m, 2H). $^{13}\text{C-NMR}$ (101 MHz, CDCl₃) δ 165.7, 165.6, 165.6, 165.5, 165.2, 133.7, 133.5, 133.3, 132.9, 132.3, 130.1, 130.0, 129.9, 129.9, 129.8, 129.6, 129.4, 129.2, 129.0, 129.0, 128.9, 128.8, 128.6, 128.6, 128.5, 128.4, 128.2, 125.4, 102.3, 102.2, 102.0, 86.7, 80.7, 80.5, 80.3, 74.9, 73.3, 73.2, 73.0, 72.1, 71.3, 71.1, 69.4, 67.2, 63.6, 63.6, 62.9. **HRMS (MALDI)** m/z calcd for C₆₈H₆₂O₂₂S (M+Na⁺) 1285.3346, found 1285.3362.



(39) Phenyl 2,3,4-tri-*O*-benzoyl- β -D-xylopyranosyl-(1 \rightarrow 4)-3-*O*-benzoyl-2-*O*-levulinoyl-1-thio- β -D-xylopyranoside This

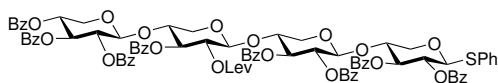
compound was synthesized according to General Procedure II in 89% yield after purification via flash chromatography (toluene/heptane/ethyl acetate 2:4:3). $[\alpha] = 43.6$ (c 0.38, CHCl₃). $^1\text{H-NMR}$ (400 MHz, CDCl₃) δ 8.09 – 7.88 (m, 8H, ArH), 7.63 – 7.28 (m, 17H, ArH), 5.64 (t, $J = 6.6$ Hz, 1H, H-3'), 5.50 (t, $J = 8.2$ Hz, 1H, H-3), 5.22 (dd, $J = 6.6, 4.9$ Hz, 1H, H-2'), 5.09 (t, $J = 8.5$ Hz, 1H, H-2), 5.10 – 5.06 (m, 1H, H-4'), 4.91 (d, $J = 4.8$ Hz, 1H, H-1'), 4.83 (d, $J = 8.6$ Hz, 1H, H-1), 4.19 (dd, $J = 12.0, 4.9$ Hz, 1H, H-5), 4.09 – 3.95 (m, 2H, H-4, H-5'), 3.45 (dd, $J = 12.0, 9.1$ Hz, 1H, H-5), 3.43 (dd, $J = 12.5, 6.1$ Hz, 1H, H-5'), 2.72 – 2.41 (m, 4H, 2xCH₂), 2.08 (s, 3H, CH₃). $^{13}\text{C-NMR}$ (101 MHz, CDCl₃) δ 205.9 (Lev, C(O)), 171.3 (Lev, C(O)), 165.5 (Bz), 165.4 (Bz), 165.3 (Bz), 165.0 (Bz), 133.5, 133.5, 133.5, 132.8, 132.4, 130.0, 130.0, 129.9, 129.9, 129.5, 129.3, 129.1, 129.0, 128.6, 128.6,

128.5, 128.5, 128.2, 99.7 (C-1'), 86.4 (C-1), 75.2 (C-4), 73.4 (C-3), 70.3 (C-2), 70.1 (C-2'), 69.5 (C-3'), 68.5 (C-4'), 66.0 (C-5), 60.8 (C-5'), 37.8 (CH₂), 29.7 (CH₂), 28.0 (CH₂). **HRMS (MALDI)** *m/z* calcd for C₄₉H₄₄O₁₄S (M+Na⁺) 911.2343, found 911.2362.



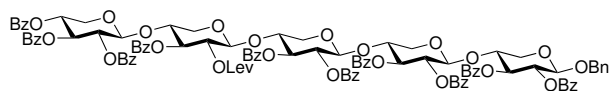
(40) Phenyl 2,3,4-tri-*O*-benzoyl- β -D-xylopyranosyl-(1 \rightarrow 4)-3-*O*-benzoyl-2-*O*-levulinoyl- β -D-xylopyranosyl-(1 \rightarrow 4)-2,3-di-*O*-benzoyl-1-thio- β -D-xylopyranoside

This compound was synthesized according to General Procedure II in 66% yield after purification via flash chromatography (toluene/heptane/ethyl acetate 2:1:1). $[\alpha] = 43.6$ (c 0.38, CHCl₃). **¹H-NMR** (400 MHz, CDCl₃) δ 8.09 – 7.88 (m, 8H, ArH), 7.63 – 7.28 (m, 17H, ArH), 5.64 (t, *J* = 6.6 Hz, 1H, H-3'), 5.50 (t, *J* = 8.2 Hz, 1H, H-3), 5.22 (dd, *J* = 6.6, 4.9 Hz, 1H, H-2'), 5.09 (t, *J* = 8.5 Hz, 1H, H-2), 5.10 – 5.06 (m, 1H, H-4'), 4.91 (d, *J* = 4.8 Hz, 1H, H-1'), 4.83 (d, *J* = 8.6 Hz, 1H, H-1), 4.19 (dd, *J* = 12.0, 4.9 Hz, 1H, H-5), 4.09 – 3.95 (m, 2H, H-4, H-5'), 3.45 (dd, *J* = 12.0, 9.1 Hz, 1H, H-5), 3.43 (dd, *J* = 12.5, 6.1 Hz, 1H, H-5'), 2.72 – 2.41 (m, 4H, 2xCH₂), 2.08 (s, 3H, CH₃). **¹³C-NMR** (101 MHz, CDCl₃) δ 205.9 (Lev, C(O)), 171.3 (Lev, C(O)), 165.5 (Bz), 165.4 (Bz), 165.3 (Bz), 165.0 (Bz), 133.5, 133.5, 133.5, 132.8, 132.4, 130.0, 130.0, 129.9, 129.9, 129.5, 129.3, 129.1, 129.0, 128.6, 128.6, 128.5, 128.5, 128.2, 99.7 (C-1'), 86.4 (C-1), 75.2 (C-4), 73.4 (C-3), 70.3 (C-2), 70.1 (C-2'), 69.5 (C-3'), 68.5 (C-4'), 66.0 (C-5), 60.8 (C-5'), 37.8 (CH₂), 29.7 (CH₂), 28.0 (CH₃). **HRMS (MALDI)** *m/z* calcd for C₄₉H₄₄O₁₄S (M+Na⁺) 911.2343, found 911.2362.



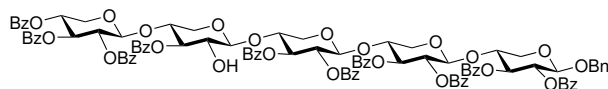
(41) Phenyl 2,3,4-tri-*O*-benzoyl- β -D-xylopyranosyl-(1 \rightarrow 4)-3-*O*-benzoyl-2-*O*-levulinyl-

β -D-xylo-pyranosyl-(1 \rightarrow 4)-2,3-di-*O*-benzoyl- β -D-xylopyranosyl-(1 \rightarrow 4)-2,3-di-*O*-benzoyl-1-thio- β -D-xylopyranoside This compound was synthesized according to General Procedure II. (toluene/heptane/ethyl acetate 3:3:2). $[\alpha]_D^{20} = -42.1$ (c 0.38, CHCl₃). ¹H-NMR (400 MHz, CDCl₃) δ 8.23 – 7.78 (m, 15H, ArH), 7.72 – 6.98 (m, 30H, ArH), 5.63 (t, $J = 7.9$ Hz, 1H, H-3), 5.57 (t, $J = 6.7$ Hz, 1H, H-3''), 5.46 (t, $J = 7.7$ Hz, 1H, H-3'), 5.35 – 5.25 (m, 2H, H-2, H-3''), 5.15 (dd, $J = 7.9, 6.0$ Hz, 1H, H-2'), 5.11 (dd, $J = 9.1, 4.2$ Hz, 1H, H-2''), 5.03 (td, $J = 6.4, 4.1$ Hz, 1H, H-4''), 4.99 (d, $J = 8.0$ Hz, 1H, H-1), 4.83 (dd, $J = 8.7, 6.8$ Hz, 1H, H-2'), 4.78 (d, $J = 6.1$ Hz, 1H, H-1'), 4.66 (d, $J = 4.9$ Hz, 1H, H-1''), 4.35 (d, $J = 6.8$ Hz, 1H, H-1'), 4.18 (dd, $J = 12.1, 4.7$ Hz, 1H, H-5), 4.07 – 3.99 (m, 1H, H-4), 3.97 (dd, $J = 12.4, 3.9$ Hz, 1H, H-5''), 3.79 – 3.63 (m, 3H, H-4'', H-4', H-5'), 3.49 (dd, $J = 8.5, 5.2$ Hz, 1H, H-5), 3.46 (t, $J = 6.1$ Hz, 1H, H-5''), 3.33 (dd, $J = 12.4, 6.3$ Hz, 1H, H-5''), 3.28 (dd, $J = 11.8, 8.0$ Hz, 1H, H-5'), 3.02 (dd, $J = 12.2, 9.1$ Hz, 1H, H-5''), 2.63 – 2.45 (m, 2H, CH₂), 2.44 – 2.28 (m, 2H, CH₂), 2.06 (s, 3H, CH₃). ¹³C-NMR (101 MHz, CDCl₃) δ 205.8 (Lev), 171.2 (Lev), 165.6 (Bz), 165.5 (Bz), 165.5 (Bz), 165.4 (Bz), 165.3 (Bz), 165.3 (Bz), 165.2 (Bz), 165.0 (Bz), 133.5, 133.5, 133.4, 133.4, 133.3, 133.2, 132.9, 132.6, 130.1, 130.0, 130.0, 129.9, 129.9, 129.8, 129.8, 129.7, 129.6, 129.5, 129.5, 129.4, 129.3, 129.2, 129.1, 129.0, 128.6, 128.6, 128.5, 128.5, 128.4, 128.4, 128.1, 100.8 (C-1'), 100.6 (C-1''), 99.5 (C-1'''), 86.7 (C-1), 75.4 (C-4), 75.3 (C-4'), 75.1 (C-4''), 73.0 (C-3), 72.5 (C-3''), 71.9 (C-3'), 71.4 (C-2'), 71.3 (C-2''), 70.5 (C-2), 70.1 (C-2''), 69.6 (C-3'''), 68.6 (C-4''), 65.7 (C-5), 62.4 (C-5''), 62.3 (C-5'), 60.9 (C-5'''), 37.8 (CH₂), 29.8 (CH₂), 27.9 (CH₃). HRMS (MALDI) m/z calcd for C₈₇H₇₆O₂₆S (M+Na⁺) 1591.4238, found 1591.4254.



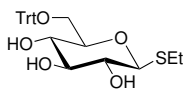
(42) Benzyl 2,3,4-tri-
O-benzoyl- β -D-xy-
lopyranosyl-(1 \rightarrow 4)-
3-O-benzoyl-2-O-

levulinyl- β -D-xylopyranosyl-(1 \rightarrow 4)-2,3-di-O-benzoyl- β -D-xylopyranosyl-(1 \rightarrow 4)-2,3-di-O-benzoyl- β -D-xylopyranosyl-(1 \rightarrow 4)-2,3-di-O-benzoyl- β -D-xylopyranoside This compound was synthesized according to General Procedure II. (toluene/heptane/ethyl acetate 3:3:2), $[\alpha]_D^{20} = -43.0$ (c 0.10, CHCl_3), $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.00 – 7.82 (m, 20H, ArH), 7.60 – 7.47 (m, 6H, ArH), 7.46 – 7.12 (m, 29H, ArH), 5.56 (t, $J = 6.7$ Hz, 1H, H-3'''), 5.54 – 5.50 (dd, $J = 7.5, 8.1$ Hz, 1H, H-3), 5.47 (t, $J = 8.1$ Hz, 1H, H-3'), 5.39 (t, $J = 8.0$ Hz, 1H, H-3''), 5.29 (dd, $J = 8.4, 6.6$ Hz, 1H, H-2), 5.28 (t, $J = 8.3$ Hz, 1H, H-3''), 5.14 (dd, $J = 8.4, 6.5$ Hz, 1H, H-2'), 5.09 (dd, $J = 6.9, 5.1$ Hz, 1H, H-2'''), 5.04 (dd, $J = 8.2, 6.3$ Hz, 1H, H-2''), 5.02 (m, 1H, H-4'''), 4.81 (dd, $J = 8.5, 6.7$ Hz, 1H, H-2''), 4.79 (d, $J = 12.2$ Hz, 1H, OCH_2Ph), 4.69 (d, $J = 6.3$ Hz, 1H, H-1'), 4.65 (d, $J = 5.0$ Hz, 1H, H-1'''), 4.63 (d, $J = 6.7$ Hz, 1H, H-1), 4.55 (d, $J = 12.3$ Hz, 1H, OCH_2Ph), 4.53 (d, $J = 6.1$ Hz, 1H, H-1''), 4.32 (d, $J = 6.7$ Hz, 1H, H-1''), 4.00 – 3.92 (m, 3H, H-4, H-5, H-5'''), 3.80 – 3.63 (m, 3H, H-4', H-4'', H-4'''), 3.59 (dd, $J = 12.2, 4.8$ Hz, 1H, H-5''), 3.45 (dd, $J = 12.2, 4.6$ Hz, 1H, H-5'), 3.44 – 3.38 (m, 1H, H-5'), 3.36 (dd, $J = 12.2, 8.9$ Hz, 1H, H-5), 3.32 (dd, $J = 12.3, 6.2$ Hz, 1H, H-5'''), 3.19 (dd, $J = 12.1, 8.4$ Hz, 1H, H-5''), 3.08 (dd, $J = 12.2, 8.6$ Hz, 1H, H-5'), 2.99 (dd, $J = 12.2, 9.0$ Hz, 1H, H-5'''), 2.60 – 2.45 (m, 2H, CH_2), 2.42 – 2.28 (m, 2H, CH_2), 2.05 (s, 3H, CH_3). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 205.7 (Lev), 171.1 (Lev), 165.5 (Bz), 165.4 (Bz), 165.3 (Bz), 165.3 (Bz), 165.3 (Bz), 165.2 (2xBz), 165.0 (Bz), 164.9 (Bz), 164.9 (Bz), 136.8, 133.4, 133.4, 133.3, 133.3, 133.2, 133.1, 133.0, 133.0, 129.9, 129.9, 129.9, 129.8, 129.7, 129.7, 129.6, 129.6, 129.5, 129.5, 129.4, 129.3, 129.3, 129.2, 129.0, 128.9, 128.5, 128.5, 128.4, 128.4, 128.4, 128.3, 128.3, 128.2, 128.2, 127.8, 127.8, 101.0 (C-1'), 100.4 (C-1'''), 100.4 (C-1''), 99.4 (C-1'''), 99.3 (C-1), 76.0 (C-4), 75.2 (C-4''), 75.1 (C-4'), 74.9 (C-4'''), 72.4 (C-3'''), 72.1 (C-3), 72.0 (C-3'), 71.9 (C-3''), 71.4 (C-2'''), 71.3 (C-2'), 71.1 (C-2''), 71.0 (C-2), 70.3 (OCH_2Ph), 70.0 (C-2'''), 69.5 (C-3'''), 68.5 (C-4'''), 62.6 (C-5), 62.3 (C-5''), 62.2 (C-5'), 60.7 (C-5'''), 37.7 (CH_2), 29.6 (CH_2), 27.7 (CH_3). **HRMS (MALDI)** m/z calcd for $\text{C}_{107}\text{H}_{94}\text{O}_{33}$ ($\text{M}+\text{Na}^+$) 1930.5603, found 1930.5619.



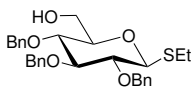
(43) Benzyl 2,3,4-tri-*O*-benzoyl- β -D-xylopyranosyl-(1 \rightarrow 4)-3-*O*-benzo-

yl- β -D-xylopyranosyl-(1 \rightarrow 4)-2,3-di-*O*-benzoyl- β -D-xylopyranosyl-(1 \rightarrow 4)-2,3-di-*O*-benzoyl- β -D-xylopyranoside This compound was synthesized according to General Procedure V in 98% yield. $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.06 – 7.99 (m, 2H), 8.00 – 7.83 (m, 18H), 7.61 – 7.48 (m, 8H), 7.49 – 7.27 (m, 21H), 7.25 – 7.13 (m, 6H), 5.65 (t, J = 6.7 Hz, 1H, H-3'''), 5.56-5.41 (m, 2H, H-3, H-3'), 5.41 (t, J = 8.0 Hz, 1H, H-3''), 5.33 – 5.29 (m, 1H, H-2), 5.24 – 5.14 (m, 3H, H-2''', H-3'', H-2'), 5.13-5.05 (m, 2H, H-4''', H-2''), 4.80 (d, J = 12.3 Hz, 1H, $-\text{OCH}_2\text{-Ph}$), 4.73 (d, J = 5.3 Hz, 1H, H-1'''), 4.71 (d, J = 6.8 Hz, 1H, H-1'), 4.64 (d, J = 6.6 Hz, 1H, H-1), 4.55 (d, J = 12.2 Hz, 1H, $\text{O-CH}_2\text{-Ph}$), 4.55 (d, J = 6.2 Hz, 1H, H-1''), 4.28 (d, J = 5.7 Hz, 1H), 4.28 (d, J = 5.7 Hz, 1H, H-1'''), 4.15 (dd, J = 12.4, 4.0 Hz, 1H, H-5'''), 4.02 – 3.95 (m, 2H, H-4, H-5), 3.80 – 3.71 (m, 3H, H-4''', H-4'', H-4'), 3.63 – 3.58 (m, 2H, H-5'', H-5'), 3.48 – 3.41 (m, 3H, H-5''', H-2'', H-5''), 3.43 – 3.32 (m, 1H, H-5), 3.18 – 3.04 (m, 3H, H-5'', H-5'', H-5'), 2.85 (d, J = 6.0 Hz, 1H, $-\text{OH}$). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ = 166.1 (Bz), 165.6 (Bz), 165.4 (Bz), 165.4 (Bz), 165.4 (Bz), 165.3 (Bz), 165.2 (Bz), 165.1 (Bz), 164.9 (Bz), 136.9, 133.5, 133.5, 133.4, 133.3, 133.2, 133.2, 133.0, 130.0, 130.0, 129.9, 129.9, 129.8, 129.8, 129.7, 129.6, 129.5, 129.5, 129.3, 129.2, 129.1, 128.9, 128.6, 128.5, 128.4, 128.4, 128.3, 127.9, 127.8, 101.8 (C-1'''), 101.1 (C-1'), 100.6 (C-1''), 99.4 (C-1), 99.0 (C-1'''), 76.1 (C-4), 75.4 (C-4'), 74.1 (C-4''), 73.2 (C-3'''), 72.3 (C-3), 72.2 (C-3'), 71.8 (C-3''), 71.5 (C-2'), 71.2 (C-2''), 71.1 (C-2''', C-2), 70.4 ($\text{CH}_2\text{-Ph}$), 70.2 (C-2'''), 69.5 (C-3'''), 68.6 (C-4'''), 62.7 (C-5), 62.4 (C-5'), 62.0 (C-5''), 61.3 (C-5'''), 61.0 (C-5'''). **HRMS (MALDI)** m/z calcd for $\text{C}_{102}\text{H}_{88}\text{O}_{31}$ ($\text{M}+\text{Na}^+$) 1831.5202, found 1831.5119



(48) Ethyl 6-*O*-trityl-1-thio-β-D-glucopyranoside Ethyl 1-thio-β-D-glucopyranoside (22.0 g, 0.0891 mol) was dissolved in 120 ml of pyridine and trityl chloride (32.8 g, 0.118 mol) was added.

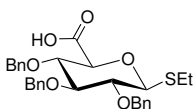
The mixture was stirred at 90 °C for 3 hours then cooled to room temperature and diluted with ethyl acetate (300 ml). The mixture was washed with 1 M HCl (2x300 ml) and the organic layer isolated, dried over Na₂SO₄, filtered and concentrated. The crude material was purified by flash chromatography (6 heptane : 4 acetone, *R_f* 0.23) to yield **48** (31.8 g, 70%) as a white amorphous solid. ¹H-NMR (400 MHz, CDCl₃) δ 7.44 – 7.22 (m, 15H, ArH), 4.34 (d, *J* = 9.6 Hz, H- 1), 3.66 – 3.48 (m, 2H, H-3, H-4), 3.41 – 3.29 (m, 4H, H-2, H-5, H-6, H-6), 2.91 (s, 1H, OH), 2.88 (s, 1H, OH), 2.84 – 2.59 (m, 2H, SCH₂CH₃), 2.54 (d, *J* = 1.3 Hz, 1H, OH), 1.27 (t, *J* = 7.4 Hz, 3H, SCH₂CH₃). ¹³C-NMR (101 MHz, CDCl₃) δ 143.6, 128.6, 128.0, 127.3, 85.9 (C-1), 77.8, 77.8, 72.3, 72.3, 64.7 (C-6), 24.4 (SCH₂CH₃), 22.8 (SCH₂CH₃). The data are in accordance with literature.²²²



(50) Ethyl 2,3,4-tri-*O*-benzyl-1-thio-β-D-glucopyranoside Triol **48** (22.6 g, 484 mmol) was dissolved in anhydrous DMF (200 ml) and NaH (9.68 g, 242 mmol, 60% oil dispersion) was added. The reaction

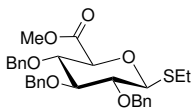
mixture was cooled to 0 °C and stirred for 10 minutes. TBAI (1.25 g, 3.39 mmol) and BnBr (28.7 ml, 242 mmol) were added slowly to the mixture. The reaction was stirred for 16 hours at room temperature then the excess of NaH and BnBr was quenched with methanol. The mixture was diluted with Et₂O (650 ml) and washed with water (2x400 ml). The organic phases were collected and dried over Na₂SO₄, filtered and concentrated under reduced pressure. The crude product was dissolved directly in methanol (450 ml) and concentrated H₂SO₄ (4.5 ml) was added. The reaction mixture was stirred 1 hour then Na₂CO₃ (38.1 g) was added to neutralize the reaction. After 2 hours the salts were filtered off and the filtrate was diluted with dichloromethane (500 ml) and washed with water (2x450 ml). The organic layers were combined and dried over Na₂SO₄. Filtration and evaporation of the solvent under vacuum gave the crude material which was purified by column chromatography (6 heptane : 4 acetone, *R_f* 0.38) to yield **50** (13.3 g, 56%). ¹H-NMR (400 MHz, CDCl₃) δ 7.42 – 7.21 (m, 15H, ArH),

4.93 (d, $J = 10.9$ Hz, 1H, OCH₂Ph), 4.92 (d, $J = 10.2$ Hz, 1H, OCH₂Ph), 4.90 – 4.83 (m, 2H, OCH₂Ph), 4.75 (d, $J = 10.2$ Hz, 1H, OCH₂Ph), 4.66 (d, $J = 10.9$ Hz, 1H, OCH₂Ph), 4.51 (d, $J = 9.8$ Hz, 1H, H-1), 3.87 (dd, $J = 12.0, 2.6$ Hz, 1H, H-6), 3.76 – 3.65 (m, 2H, H-3, H-6), 3.58 (t, $J = 9.4$ Hz, 1H, H-4), 3.41 (t, $J = 9.5$ Hz, 1H, H-2), 3.40 – 3.34 (m, 1H, H-5), 2.84 – 2.66 (m, 2H, SCH₂CH₃), 1.33 (t, $J = 7.4$ Hz, 3H, SCH₂CH₃). ¹³C-NMR (101 MHz, CDCl₃) δ 138.4, 137.9, 128.5, 128.5, 128.4, 128.3, 128.1, 128.0, 127.9, 127.8, 127.7, 86.5 (C-3), 85.3 (C-1), 81.8 (C-2), 79.3 (C-5), 77.7 (C-4), 75.8 (OCH₂Ph), 75.6 (OCH₂Ph), 75.2 (OCH₂Ph), 62.2 (C-6), 25.2 (SCH₂CH₃), 15.2 (SCH₂CH₃). The data are in accordance with the literature.²⁰⁹



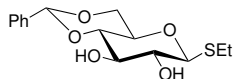
(52) Ethyl 2,3,4-tri-*O*-benzyl-1-thio- β -D-glucopyranosiduronic acid The alcohol **50** (0.500 g, 1.01 mmol) was dissolved in a solvent mixture CH₂Cl₂/H₂O (2:1, 5 ml) and stirred vigorously. TEMPO (0.032 g, 0.202 mmol) and PhI(OAc)₂ (0.815 g, 2.53 mmol) were

added to the mixture. Full conversion of the starting material was observed after 50 minutes with TLC and the remaining oxidant was quenched using Na₂S₂O₃ (20 ml, 10% solution). The water phase was extracted with ethyl acetate (2x20 ml), then organic layers were dried over NaSO₄, filtered and the solvent evaporated under vacuum. The carboxylic acid **52** (0.416 g, 81%) was isolated by flash chromatography (heptane/ethyl acetate/AcOH 7:3:0.5, R_f 0.40). ¹H-NMR (400 MHz, CDCl₃) δ 7.40 – 7.20 (m, 15H, ArH), 4.90 (d, $J = 10.3$ Hz, 1H, OCH₂Ph), 4.86 (d, $J = 11.1$ Hz, 1H, OCH₂Ph), 4.81 (d, $J = 11.1$ Hz, 1H, OCH₂Ph), 4.78 (d, $J = 10.7$ Hz, 1H, OCH₂Ph), 4.72 (d, $J = 10.3$ Hz, 1H, OCH₂Ph), 4.66 (d, $J = 10.7$ Hz, 1H, OCH₂Ph), 4.59 (d, $J = 9.7$ Hz, 1H, H-1), 3.98 (d, $J = 9.1$ Hz, 1H, H-5), 3.83 (t, $J = 8.9$ Hz, 1H, H-4), 3.72 (t, $J = 8.4$ Hz, 1H, H-3), 3.49 (dd, $J = 9.4, 8.3$ Hz, 1H, H-2), 2.84 – 2.67 (m, 2H, SCH₂CH₃), 1.32 (t, $J = 7.4$ Hz, 3H, SCH₂CH₃). ¹³C-NMR (101 MHz, CDCl₃) δ 171.4 (COOH), 138.0, 137.7, 137.3, 128.5, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 85.5 (C-1), 85.0 (C-3), 81.0 (C-2), 78.6 (C-4), 77.2 (C-5), 75.6 (OCH₂Ph), 75.4 (OCH₂Ph), 75.0 (OCH₂Ph), 25.4 (SCH₂CH₃), 15.0 (SCH₂CH₃). The data are in accordance with literature.²¹²



(53) Methyl (ethyl 2,3,4-tri-*O*-benzyl-1-thio- β -D-glucopyranoside) uronate

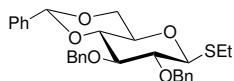
Glucuronic acid **52** (0.200 g, 0.393 mmol) was dissolved in a solvent mixture CH₃OH/toluene (1:1, 6 ml) and a solution of Me₃SiCHN₂ (0.77 ml, 2M in hexane) was slowly added to the mixture. After 3 h TLC showed full conversion of the starting material and the excess reagent was quenched with acetic acid (2 ml). The reaction mixture was concentrated and the crude material was purified by column chromatography (heptane/ethyl acetate 8:2, *R_f* 0.36) to yield the methyl ester **53** as a white amorphous solid (0.189 g, 92%). ¹H-NMR (400 MHz, CDCl₃) δ 7.44 – 6.98 (m, 15H, ArH), 4.92 (d, *J* = 10.2 Hz, 1H, OCH₂Ph), 4.91 (d, *J* = 10.9 Hz, 1H, OCH₂Ph), 4.85 (d, *J* = 10.9 Hz, 1H, OCH₂Ph), 4.78 (d, *J* = 10.8 Hz, 1H, OCH₂Ph), 4.74 (d, *J* = 10.2 Hz, 1H, OCH₂Ph), 4.61 (d, *J* = 10.8 Hz, 1H, OCH₂Ph), 4.50 (d, *J* = 9.7 Hz, 1H, H-1), 3.89 (d, *J* = 9.7 Hz, 1H, H-5), 3.84 (t, *J* = 9.2 Hz, 1H, H-4), 3.72 (s, 3H, OCH₃), 3.71 (m, 1H, H-3), 3.40 (t, *J* = 9.2 Hz, 1H, H-2), 2.76 – 2.59 (m, 2H, SCH₂CH₃), 1.24 (t, *J* = 7.3 Hz, 3H, SCH₂CH₃). ¹³C-NMR (101 MHz, CDCl₃) β 168.7 (C(O)-OCH₃), 138.3, 137.8, 128.5, 128.4, 128.3, 128.0, 127.9, 127.9, 129.8, 127.8, 85.9 (C-1), 85.8 (C-3), 81.2 (C-2), 79.3 (C-4), 78.1 (C-5), 75.9 (OCH₂Ph), 75.6 (OCH₂Ph), 75.1 (OCH₂Ph), 52.5 (OCH₃), 25.2 (SCH₂CH₃), 15.0 (SCH₂CH₃). The data are in accordance with literature.²¹²



(54) Ethyl 4,6-*O*-benzylidene-1-thio- β -D-glucopyranoside

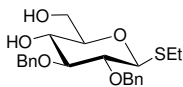
Ethyl 1-thio- β -D-glucopyranoside (8.26 g, 36.8 mmol) was dissolved in anhydrous CH₃CN (250 ml) together with PhCH(OMe)₂ (16.6 ml, 110 mmol). CSA (4.3 g, 18.4 mmol) was added and the reaction was stirred at 22 °C for 24 h. The mixture was neutralized with Et₃N (3.6 ml, 25.8 mmol), filtered and the solvent evaporated under reduced pressure. The crude was purified by flash chromatography to yield **54** (4.5 g, 39%) as a white amorphous solid. ¹H-NMR (400 MHz, CDCl₃) δ 7.54 – 7.44 (m, 2H, ArH), 7.42 – 7.33 (m, 3H, ArH), 5.53 (s, 1H, CHPh), 4.45 (d, *J* = 9.8 Hz, 1H, H-1), 4.34 (dd, *J* = 10.5, 4.8 Hz, 1H, H-6), 3.81 (t, *J* = 8.8 Hz, 1H, H-3), 3.76 (t, *J* = 10.1 Hz, 1H, H-6), 3.56 (t, *J* = 9.2 Hz, 1H, H-4), 3.49 (t, *J* = 9.8 Hz, 1H, H-2), 3.52 – 3.45 (m, 1H, H-5), 2.75 (qd, *J* = 7.4, 2.0 Hz, 2H, SCH₂CH₃), 1.32 (t, *J* = 7.4 Hz, 3H, SCH₂CH₃). ¹³C-NMR (101 MHz, CDCl₃) δ 136.9, 129.3, 128.4, 126.3,

101.9 (CHPh), 86.6 (C-1), 80.4 (C-4), 74.6 (C-3), 73.2 (C-2), 70.6 (C-5), 68.6 (C-6), 24.8 (SCH₂CH₃), 15.3 (SCH₂CH₃). The data are in accordance with the literature.²²³



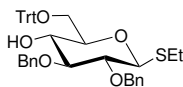
(55) Ethyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-1-thio- β -D-glucopyranoside

Diol **54** (4.2 g, 13.4 mmol) was dissolved in DMF (120 ml), then NaH (1.2 g, 51.6 mmol, 60% oil dispersion) was added and the mixture was stirred at 0 °C. BnBr (4.00 ml, 33.6 mmol) was added and the temperature was raised to room temperature. The reaction mixture was stirred for 20 h, and the remaining NaH and BnBr were quenched with methanol (35 ml) and diluted with Et₂O (200 ml). The organic phase was washed with water (2x250 ml), dried over Na₂SO₄, filtered, concentrated and purified by flash chromatography (heptane/ethyl acetate 9:1, R_f 0.24) to give **55** (4.25 g, 64%) as a colorless oil. ¹H-NMR (400 MHz, CDCl₃) δ 7.52 – 7.46 (m, 2H, ArH), 7.42 – 7.27 (m, 13H, ArH), 5.59 (s, 1H, CHPh), 4.96 (d, *J* = 11.3 Hz, 1H, OCH₂Ph), 4.89 (d, *J* = 10.2 Hz, 1H, OCH₂Ph), 4.82 (d, *J* = 10.2 Hz, 1H, OCH₂Ph), 4.81 (d, *J* = 11.3 Hz, 1H, OCH₂Ph), 4.57 (d, *J* = 9.8 Hz, 1H, H-1), 4.36 (dd, *J* = 10.5, 5.0 Hz, 1H, H-6), 3.82 (dd, *J* = 8.2, 9.3 Hz, 1H, H-3), 3.77 (t, *J* = 10.5 Hz, 1H, H-6), 3.72 (t, *J* = 9.3 Hz, 1H, H-4), 3.47 (dd, *J* = 9.7, 8.2 Hz, 1H, H-2), 3.49 – 3.40 (m, 1H, H-5), 2.85 – 2.68 (m, 2H, SCH₂CH₃), 1.33 (t, *J* = 7.4 Hz, 3H, SCH₂CH₃). ¹³C-NMR (101 MHz, CDCl₃) δ 138.4, 138.0, 137.3, 129.0, 128.4, 128.3, 128.3, 128.1, 127.9, 127.7, 126.0, 101.1 (CHPh), 85.9 (C-1), 82.8 (C-3), 81.6 (C-4), 81.3 (C-2), 76.0 (OCH₂Ph), 75.2 (OCH₂Ph), 70.2 (C-5), 68.7 (C-6), 25.2 (SCH₂CH₃), 15.1 (SCH₂CH₃). The data are in accordance with the literature.²²⁴



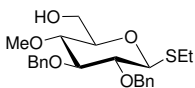
(56) Ethyl 2,3-di-*O*-benzyl-1-thio- β -D-glucopyranoside Acetal **55** (4.00 g, 8.12 mmol) was dissolved in CH_2Cl_2 (5 ml) and methanol (5 ml). After addition of *p*-toluenesulfonic acid monohydrate (0.463

g, 2.436 mmol) the mixture was left to stir for one hour. Afterwards the reaction mixture was diluted with CH_2Cl_2 and neutralized with sodium bicarbonate. The organic phase was separated and dried over MgSO_4 , filtered and concentrated. Column chromatography (toluene : ethyl acetate, 1:5) yielded the product **56** (3.02 g, 7.47 mmol, 92% yield). $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 7.39 – 7.28 (m, 15H), 4.91 – 4.81 (m, 3H), 4.73 (d, J = 10.2 Hz, 1H), 4.48 (d, J = 9.8 Hz, 1H), 3.90 (ddd, J = 12.0, 5.8, 1.7 Hz, 1H), 3.73 (ddd, J = 11.9, 7.8, 3.7 Hz, 1H), 3.62 – 3.57 (m, 1H), 3.56 (s, 3H), 3.39 – 3.33 (m, 1H), 3.30 – 3.27 (m, 2H), 2.82 – 2.67 (m, 2H), 1.99 (dd, J = 7.8, 5.9 Hz, 1H), 1.32 (t, J = 7.4 Hz, 3H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ = 138.6, 138.1, 128.6, 128.5, 128.4, 128.0, 127.9, 86.4, 85.3, 81.7, 80.0, 79.4, 75.8, 75.7, 62.3, 61.0, 25.3, 15.3. The data are in accordance with the literature.²⁰⁹



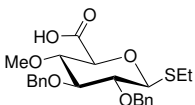
(57) Ethyl 2,3-di-*O*-benzyl-6-*O*-trityl-1-thio- β -D-glucopyranoside Trityl chloride (1.160 g, 4.16 mmol) was added to a solution of diol **56** (1.53 g, 3.78 mmol) in pyridine (8.22 ml) at room temperature.

After being stirred overnight at 55°C the mixture was diluted with toluene and concentrated. Co-evaporation twice from toluene gave a crude product, which was purified by silica gel chromatography (toluene-EtOAc 19 : 1) to yield **57** (2.177 g, 3.37 mmol, 89 % yield) $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 7.40 – 7.37 (m, 6H), 7.34 – 7.31 (m, 2H), 7.28 – 7.20 (m, 14H), 7.18 – 7.14 (m, 3H), 4.85 (dd, J = 10.7, 8.0 Hz, 2H), 4.71 (dd, J = 19.2, 10.8 Hz, 2H), 4.44 (d, J = 9.4 Hz, 1H), 3.62 – 3.56 (m, 1H), 3.46 – 3.28 (m, 5H), 2.83 – 2.64 (m, 2H), 2.41 (d, J = 2.3 Hz, 1H), 1.30 (t, J = 7.4 Hz, 3H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ = 143.8, 128.8, 128.7, 128.6, 128.5, 128.1, 128.0, 128.0, 127.3, 87.2, 86.2, 84.9, 81.5, 78.1, 75.7, 75.6, 72.4, 64.5, 24.9, 15.4. 15.2 (SEt), 24.7 (SEt). The data are in accordance with the literature.²¹⁰



(58) Ethyl 2,3-di-*O*-benzyl-4-*O*-methyl-1-thio- β -D-glucopyranoside Sodium hydride (0.579 g, 12.06 mmol), DMF (29.1 ml) was added and subsequently a solution of **57** (5.2 g, 8.04 mmol) in 2x

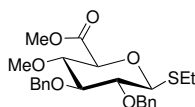
DMF (29.1 ml) dropwise at 0°C. After 1 h, a solution of methyl iodide (0.704 ml, 11.25 mmol) in DMF (29.1 ml) was added and the solution was allowed to attain room temperature. After 3 h, MeOH (5 ml) was carefully added and the mixture was diluted with toluene, washed three times with water, dried, and concentrated in vacuo. The residue was dissolved in CHCl₃- MeOH (2:1; 150 ml) and the pH was adjusted to 2 by addition of *p*-toluenesulfonic acid (PTSA). After 2h, the mixture was washed successively with water and saturated aq. NaHCO₃, dried, concentrated, and purified on a silica gel column (toluene-EtOAc 6 : 1) to give **58** (2.27 g, 5.42 mmol, 68% yield) ¹H-NMR (400 MHz, CDCl₃) δ 7.39 – 7.27 (m, 10H), 4.91 – 4.82 (m, 3H), 4.73 (d, *J* = 10.2 Hz, 1H), 4.48 (d, *J* = 9.8 Hz, 1H), 3.90 (ddd, *J* = 12.0, 5.8, 1.7 Hz, 1H), 3.73 (ddd, *J* = 11.9, 7.8, 3.7 Hz, 1H), 3.62 – 3.57 (m, 1H), 3.56 (s, 3H), 3.38 – 3.33 (m, 1H), 3.32 – 3.25 (m, 2H), 2.84 – 2.66 (m, 2H), 1.99 (dd, *J* = 7.8, 5.9 Hz, 1H), 1.32 (t, *J* = 7.4 Hz, 3H). ¹³C-NMR (101 MHz, CDCl₃) δ = 138.6, 138.1, 128.6, 128.5, 128.4, 128.0, 127.9, 86.4, 85.3, 81.7, 80.0, 79.4, 75.8, 75.7, 62.3, 61.0, 25.3, 15.3. The data are in accordance with the literature.²¹⁰



(59) Ethyl 2,3-di-*O*-benzyl-4-*O*-methyl-1-thio- β -D-glucopyranosiduronic acid

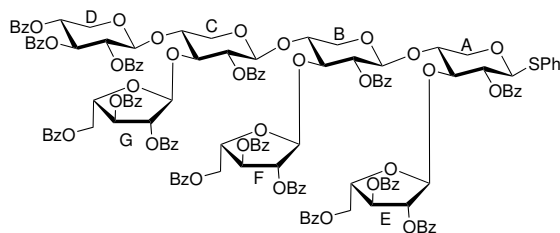
To a vigorously stirred solution of alcohol **58** (2.49 g, 5.95 mmol) in 17 ml CH₂Cl₂ and 8.5 ml H₂O was added TEMPO (0.186 g, 1.190 mmol) and phenyl-I₃-iodanediyl diacetate (4.79 g, 14.87 mmol). Stirring was allowed until TLC indicated complete conversion of the starting material to a lower running spot (approx. 45 min). The reaction mixture was quenched by the addition of 10 ml Na₂S₂O₃ solution (10% in H₂O). The mixture was then extracted twice with EtOAc (10 ml) and the combined organic layers were dried (MgSO₄), filtered and concentrated. Flash column chromatography using 5 ethyl acetate : 5 heptane + 1% AcOH afforded the pure glycuronic acid **59** (1.886 g, 4.36 mmol, 73% yield) ¹H-NMR (400 MHz, CDCl₃) δ 7.38 – 7.27 (m, 10H), 4.87 (d, *J* = 10.4 Hz, 1H,

BnO-2), 4.82 (d, $J = 11.0$ Hz, 1H, BnO-3), 4.78 (d, $J = 11.0$ Hz, 1H, BnO-3), 4.70 (d, $J = 10.4$ Hz, 1H, BnO-2), 4.57 (d, $J = 9.7$ Hz, 1H, H-1), 3.91 (d, $J = 8.9$ Hz, 1H, H-5), 3.64 (t, $J = 8.2$ Hz, 1H, H-3), 3.55 (s, 3H), 3.60 – 3.50 (m, 1H, H-4), 3.43 (dd, $J = 9.6, 7.9$ Hz, 1H, H-2), 2.83 – 2.68 (m, 2H), 1.32 (t, $J = 7.4$ Hz, 3H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) $\delta = 170.6$ (COOH), 138.1, 137.8, 128.6, 128.6, 128.4, 128.1, 128.0, 128.0, 85.5 (C-1), 84.9 (C-3), 80.8 (C-2, C-4), 76.9 (C-5), 75.6 (BnO-2), 75.5 (BnO-3), 60.8 (OMe), 25.5 (SCH_2), 15.2 (SCH_2CH_3).



(60) Methyl (ethyl 2,3-di-*O*-benzyl-4-*O*-methyl-1-thio- β -D-glucopyranosid)uronate

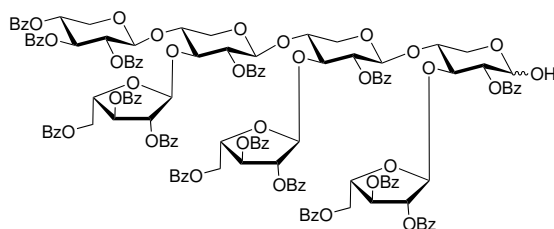
To a solution of acid **59** (1.886 g, 4.36 mmol) in toluene (14.75 ml) and methanol (4.21 ml) was added trimethylsilyldiazomethane (2.83 ml, 5.67 mmol). After 20 minutes of stirring at room temperature more trimethylsilyldiazomethane was added (0.5 eq). After 10 more minutes the mixture was concentrated to yield the methyl ester **60** (1.947 g, 4.36 mmol, 100% yield). The analytical data are in accordance with the literature.²¹⁰ $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 7.31 – 7.20 (m, 10H), 4.82 (d, $J = 10.2$ Hz, 1H), 4.80 (d, $J = 10.2$ Hz, 1H), 4.76 (d, $J = 11.0$ Hz, 1H), 4.65 (d, $J = 10.2$ Hz, 1H), 4.41 (d, $J = 9.7$ Hz, 1H), 3.74 (s, 3H), 3.71 (d, $J = 9.3$ Hz, 1H), 3.57 – 3.43 (m, 2H), 3.43 (s, 3H), 3.35 (dd, $J = 9.5, 8.6$ Hz, 1H), 2.76 – 2.59 (m, 2H), 1.23 (t, $J = 7.4$ Hz, 3H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 168.9, 138.4, 138.0, 128.6, 128.5, 128.4, 128.0, 128.0, 127.9, 85.9, 85.8, 81.3, 81.1, 78.1, 75.9, 75.7, 60.8, 52.7, 25.3, 15.1.



(61) Phenyl 2,3,4-Tri-*O*-benzoyl- β -D-xylopyranosyl-(1 \rightarrow 4)-[2,3,5-tri-*O*-benzoyl- α -L-arabinofuranosyl-(1 \rightarrow 3)]-2-*O*-benzoyl- β -D-xylopyranosyl-(1 \rightarrow 4)-[2,3,5-tri-*O*-benzoyl- α -L-arabinofuranosyl-

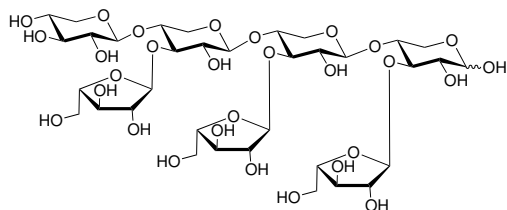
(1 \rightarrow 3)]-2-*O*-benzoyl- β -D-xylopyranosyl-(1 \rightarrow 4)-[2,3,5-tri-*O*-benzoyl- α -L-arabinofuranosyl-(1 \rightarrow 3)]-2-*O*-benzoyl-1-thio- β -D-xylopyranoside A mixture of the triol **38** (102 mg, 0.081 mmol) and acetimidate **68** (169 mg, 0.266 mmol) was co-evaporated with toluene (2×20 ml) and subjected to high vacuum for 2 h. The mixture was dissolved in anhydrous CH_2Cl_2 (20 ml) and cooled to -40°C . TMSOTf (1.459 μl , 8.07 μmol) was added and the reaction mixture was stirred at -40°C until TLC (toluene/EtOAc 20:1) showed completion of the reaction (10-30 min). The reaction mixture was quenched by addition of triethylamine (7.88 μl , 0.057 mmol) evaporated and purified by flash column chromatography (9.6 Toluene/0.4 Acetone, $R_f = 0.13$), $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.18 (d, $J = 7.2$ Hz, 2H), 8.11 – 7.87 (m, 25H), 7.65 – 7.41 (m, 28H), 7.37 – 7.16 (m, 25H), 5.72 (t, $J = 9.3$ Hz, 1H, D-3), 5.52 (d, $J = 5.6$ Hz, 1H), 5.49 (d, $J = 5.4$ Hz, 1H), 5.45 (d, $J = 4.5$ Hz, 1H), 5.43 (d, $J = 0.6$ Hz, 1H), 5.37 (d, $J = 1.0$ Hz, 1H), 5.35 (d, $J = 1.4$ Hz, 1H), 5.34 (bs, 1H, E-1), 5.30 – 5.26 (m, 2H, A-2, D-2), 5.29 (bs, 1H, F-1), 5.24 (bs, 1H, G-1), 5.16 (td, $J = 9.3, 5.5$ Hz, 1H), 5.07-4.68 (m, 11H, B-2, E-5, F-5, G-5), 4.68 (d, $J = 8.8$ Hz, 1H, A-1), 4.22 (d, $J = 8.0$ Hz, 1H, B-1), 4.11 (d, $J = 7.4$ Hz, 1H, D-1), 4.08 – 3.99 (m, 2H, A-3, A-5), 3.88 – 3.82 (m, 3H, A-3, D-5), 3.76 (td, $J = 8.8, 4.8$ Hz, 1H, A-4), 3.33 (dd, $J = 11.8, 5.1$ Hz, 1H, C-5), 3.29 – 3.23 (m, 1H, B-4), 3.22 (dd, $J = 11.8, 9.8$ Hz, 1H, D-5), 3.10 – 3.02 (m, 2H, A-5, B-5), 2.67 – 2.61 (m, 2H, C-5), 2.61 – 2.56 (m, 2H, B-5). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 166.4 (Bz), 166.4 (Bz), 166.1 (Bz), 165.9 (Bz), 165.8 (Bz), 165.7 (Bz), 165.1 (Bz), 165.1 (Bz), 165.0 (Bz), 164.7 (Bz), 164.4 (Bz), 164.2 (Bz), 133.9, 133.7, 133.5, 133.4, 133.3, 133.2, 133.2, 133.0, 132.9, 132.5, 130.4, 130.2, 130.1, 130.1, 130.1, 129.9, 129.9, 129.9, 129.8, 129.8, 129.7, 129.7, 129.6, 129.5, 129.3, 129.2, 129.2, 129.1, 129.1, 129.0, 129.0, 129.0, 128.9, 128.6, 128.5, 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 128.2, 127.9, 125.4, 106.1 (CH, F-1), 105.7 (CH, G-1), 105.6 (CH,

E-1), 100.3 (CH, B-1), 100.0 (CH, C-1), 99.8 (CH, D-1), 86.7 (C1), 82.8 (CH), 82.7 (CH), 82.5 (CH), 82.1 (CH), 81.5 (CH), 80.8 (CH), 78.3 (CH), 78.2(2xCH), 76.0(CH, A-3), 75.3(CH, C-3), 75.2(CH, B-3), 74.5 (CH, E-4), 74.2 (CH, B-4), 73.7 (CH, A-4), 73.3 (CH, D-2), 73.0 (CH, E-2), 72.2 (CH, A-2), 72.0 (CH, D-3), 71.2 (CH, D-2), 69.8 (CH, D-4), 66.0(CH₂, A-5), 64.0(CH₂), 63.9(CH₂), 63.8(CH₂), 63.1(CH₂), 63.04(CH₂), 62.96 (CH₂, D-5). **HRMS (MALDI)** *m/z* calcd for C₁₄₆H₁₂₂O₄₃S (M+Na⁺) 2617.6973, found 2618.6839.



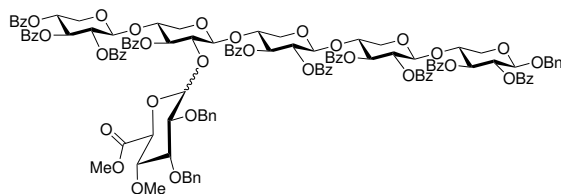
(62) 2,3,4-Tri-*O*-benzoyl- β -D-xylopyranosyl-(1 \rightarrow 4)-[2,3,5-tri-*O*-benzoyl- α -L-arabinofuranosyl-(1 \rightarrow 3)]-2-*O*-benzoyl- β -D-xylopyranosyl-(1 \rightarrow 4)-[2,3,5-tri-*O*-benzoyl- α -L-arabinofuranosyl-(1 \rightarrow 3)]-2-*O*-benzoyl- β -D-xylopyranoside

N-bromosuccinimide (41.7 mg, 0.234 mmol) was added at room temperature to a stirred solution of the phenyl thioglycoside **61** (1 equiv) in 9: 1 acetone-water (1.75 ml). Stirring was continued for a period of 60 minutes. The solvent was evaporated at room temperature until turbidity arose. The residue was dissolved in ethyl acetate washed three times with a saturated aqueous solution of sodium hydrogen carbonate, three times with water, dried over anhydrous sodium sulfate and the solvent evaporated. The product was isolated by column chromatography on silica gel (4 acetone : 6 heptane).¹³⁰ **HRMS (MALDI)** *m/z* calcd for C₁₄₀H₁₁₈O₄₄ (M+Na⁺) 2525.6888, found 2525.6699.



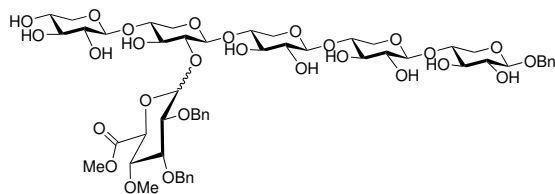
(63) β -D-xylopyranosyl-(1 \rightarrow 4)-[α -L-arabinofuranosyl-(1 \rightarrow 3)]- β -D-xylopyranosyl-(1 \rightarrow 4)-[α -L-arabinofuranosyl-(1 \rightarrow 3)] β -D-xylopyranosyl-(1 \rightarrow 4)-[α -L-arabinofuranosyl-

(1 \rightarrow 3)]-D-xylopyranoside Heptasaccharide **62** (50 mg, 0.020 mmol) was dissolved in 1 ml MeOH and 2 ml CH₂Cl₂. After addition of 1 equivalent of sodium methoxide the solution was left to stir for 45h. The solution was neutralized with Amberlite IR120 H⁺, the resin filtered off and the solvent evaporated. Purification has so far not been successful, but HRMS detection confirmed the existence of the product. **HRMS (MALDI)** m/z calcd for C₃₅H₅₈O₂₉ (M+Na⁺) 965.2956, found 965.2953



(64) Benzyl 2,3,4-Tri-*O*-benzoyl- β -D-xylopyranosyl-(1 \rightarrow 4)-[methyl 2,3,-*O*-benzyl-4-*O*-methyl-(α / β)-glucuronatyl-(1 \rightarrow 2)]-3-*O*-benzoyl- β -D-xylopyranosyl-(1 \rightarrow 4)-2,3-

O-benzoyl- β -D-xylopyranosyl-(1 \rightarrow 4)-2,3-*O*-benzoyl- β -D-xylopyranosyl-(1 \rightarrow 4)-2,3-*O*-benzoyl- β -D-xylopyranoside The donor **60** (100 mg, 0.224 mmol) was dissolved in 1 ml CH₂Cl₂. A dry solution of 0.4M bromine in CH₂Cl₂ was added (0.28 ml, 0.11 mmol). The conversion cannot be observed by TLC. Within one hour 4 equivalents of bromine were added. Afterwards, the solution is washed with sodium thiosulfate, dried and evaporated. The crude product was redissolved in toluene, mixed with 0.67 equivalents of the pentasaccharide acceptor **43** (270 mg, 0.149 mmol) and evaporated to dryness. The dry mixture is redissolved in 1 ml CH₂Cl₂ and not more than 0.1 ml diethyl ether. 100 mg of powdered molecular sieves are added and the mixture is cooled down to -30°C. Silver(I) perchlorate (55.7 mg, 0.269 mmol) is added to the solution and the mixture is stirred for 60 minutes. The mixture was quenched with 0.04 ml of triethyl amine and purified. Column chromatography (3 acetone / 7 pentane) yielded 15% of the acceptor and an inseparable mixture of both anomers of **64** (209 mg, 0.095 mmol, 64% yield, 75% b.r.s.m.). **HRMS (MALDI)** m/z calcd for C₁₂₄H₁₁₂O₃₇ (M+Na⁺) 2215.6775, found 2215.6660



(66) Benzyl β -D-xylopyranosyl-(1 \rightarrow 4)-[methyl 2,3-*O*-benzyl-4-*O*-methyl-(α / β -glucuronatyl-(1 \rightarrow 2)]- β -D-xylopyranosyl-(1 \rightarrow 4)- β -D-xylopyranosyl-(1 \rightarrow 4)-

β -D-xylopyranosyl-(1 \rightarrow 4)- β -D-xylopyranoside 150 mg of a mixture of both anomers of **64** were dissolved in 10 ml of CH₂Cl₂ / MeOH (1:1) and 1 equivalent of sodium methoxide was added. The solution was stirred over 48 hours after which it was neutralized with Amberlite IR120 H⁺. The resin was filtered off and the solvent evaporated. While purification by HPLC has so far not been successful, the existence of the product was confirmed by HRMS. **HRMS (ESI)** *m/z* calcd for C₅₄H₇₂O₂₇ (M+Na⁺) 1175.4153, found 1175.4166

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